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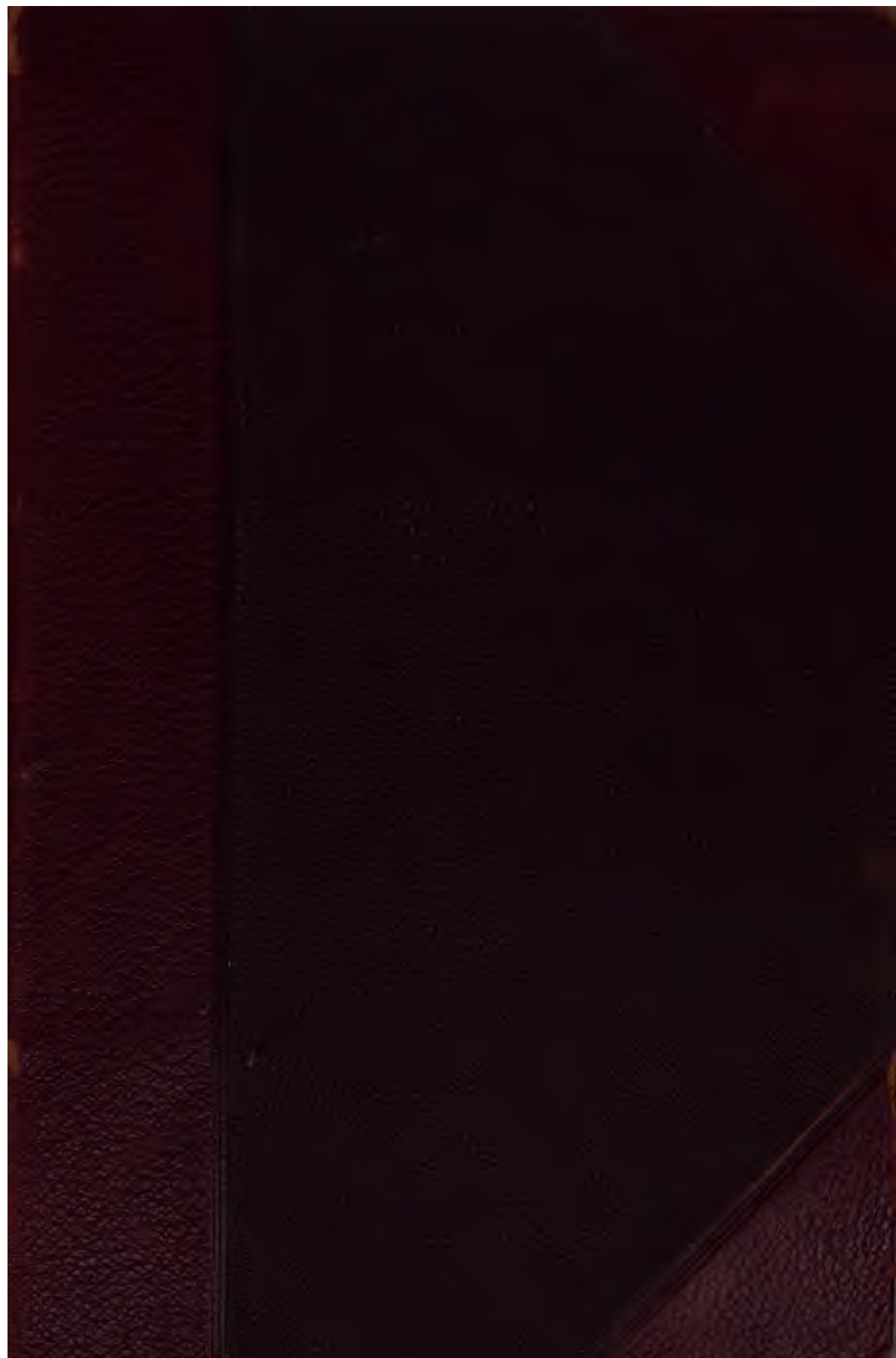
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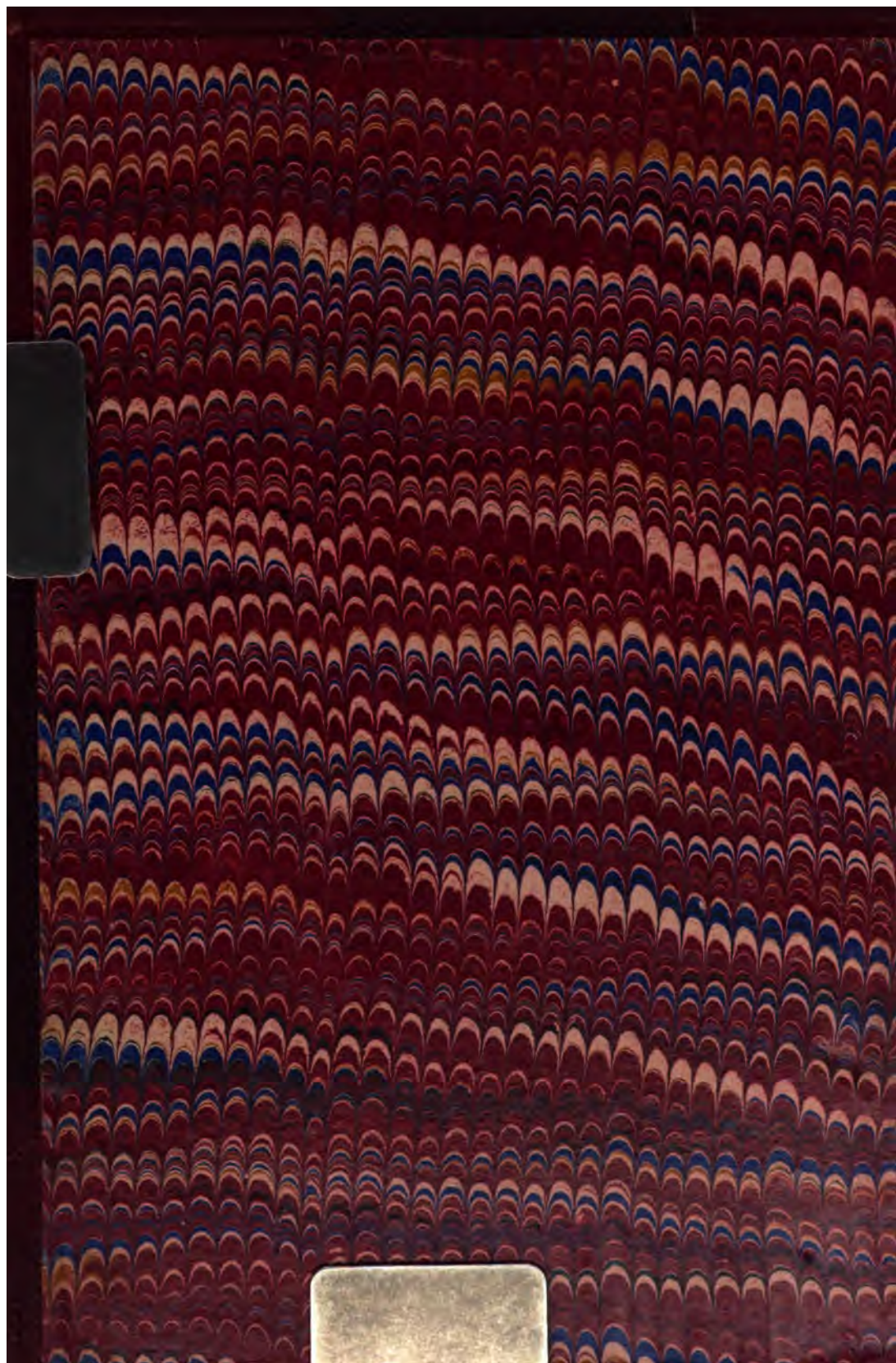
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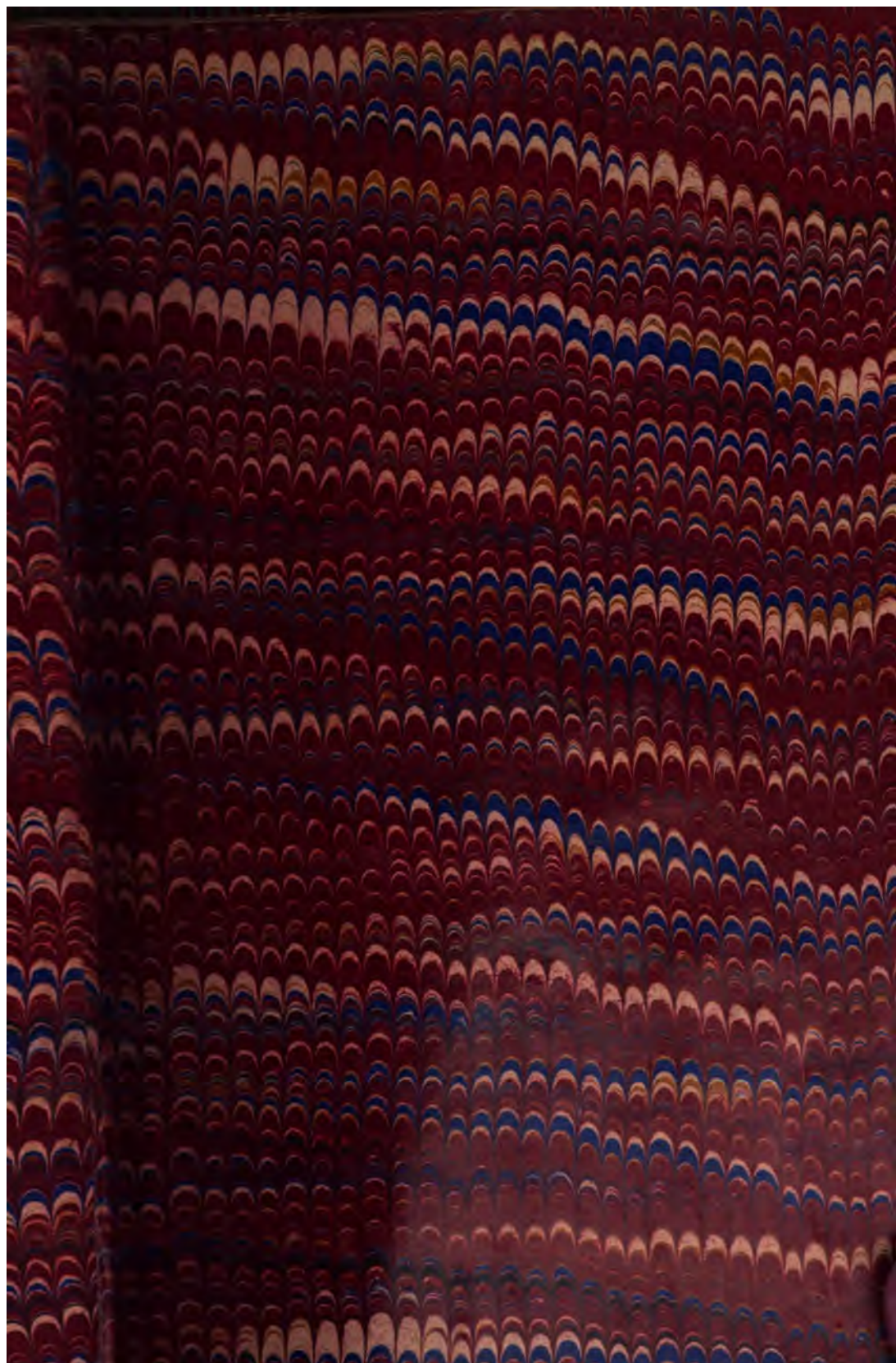
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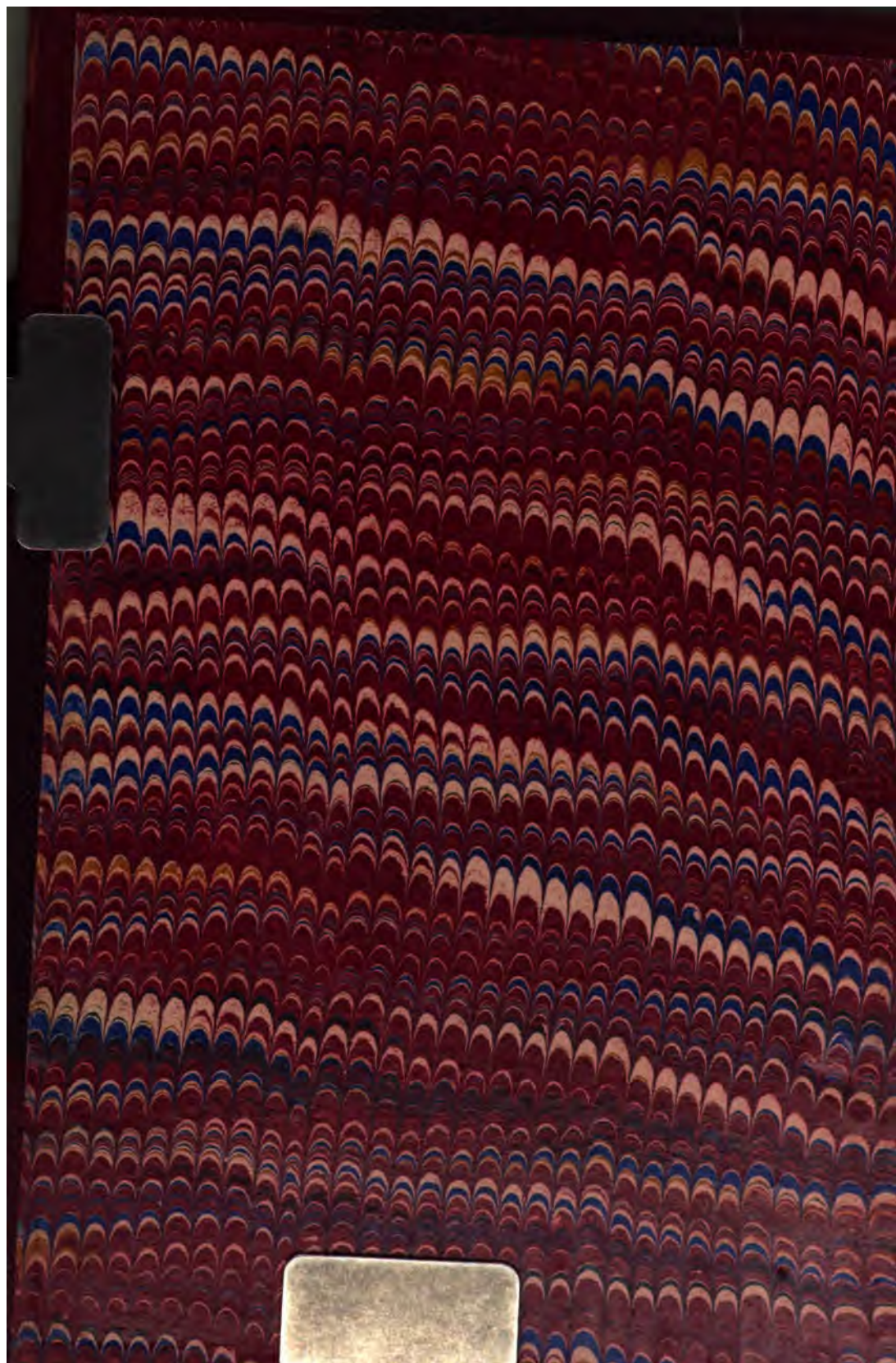




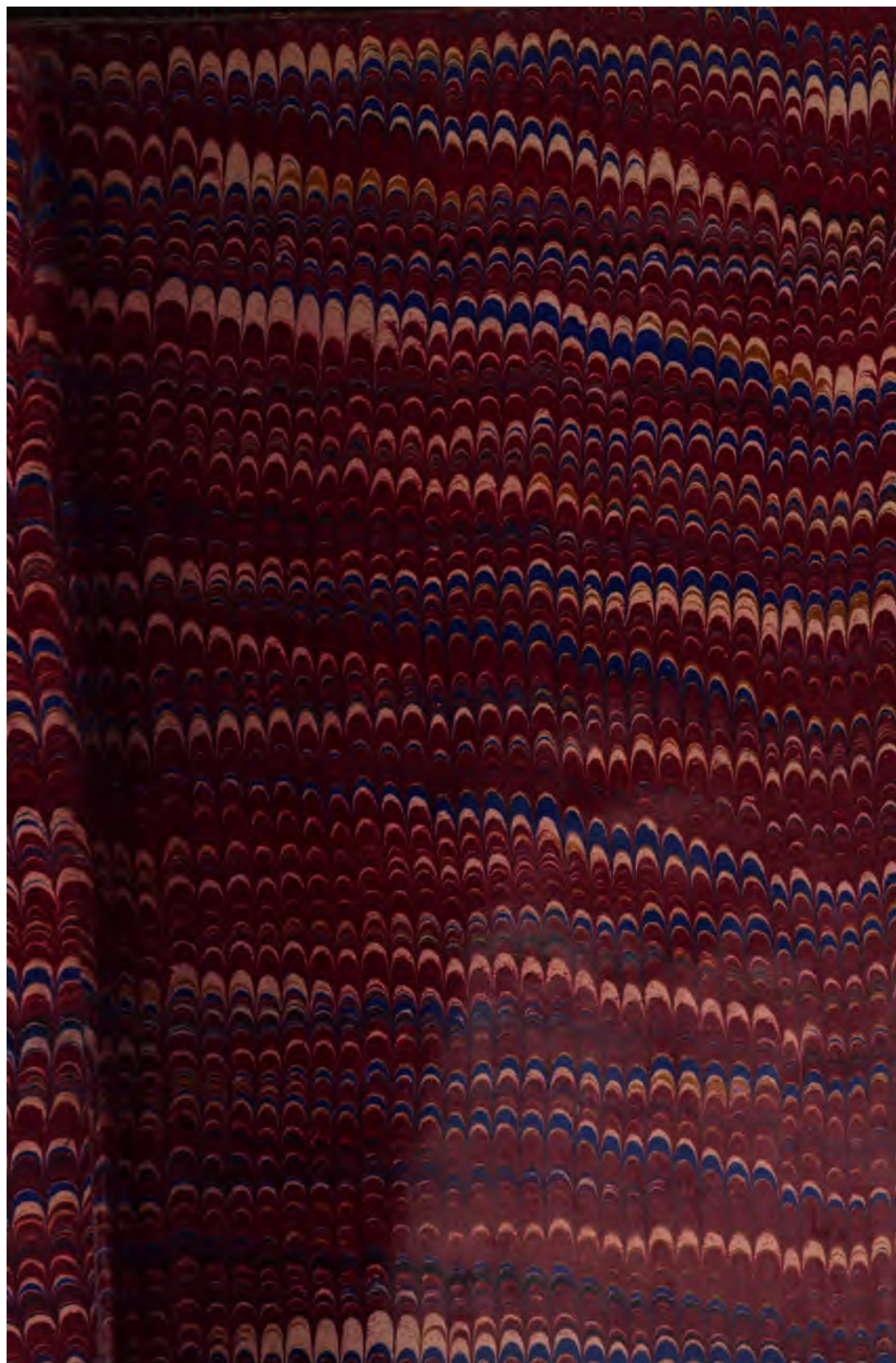




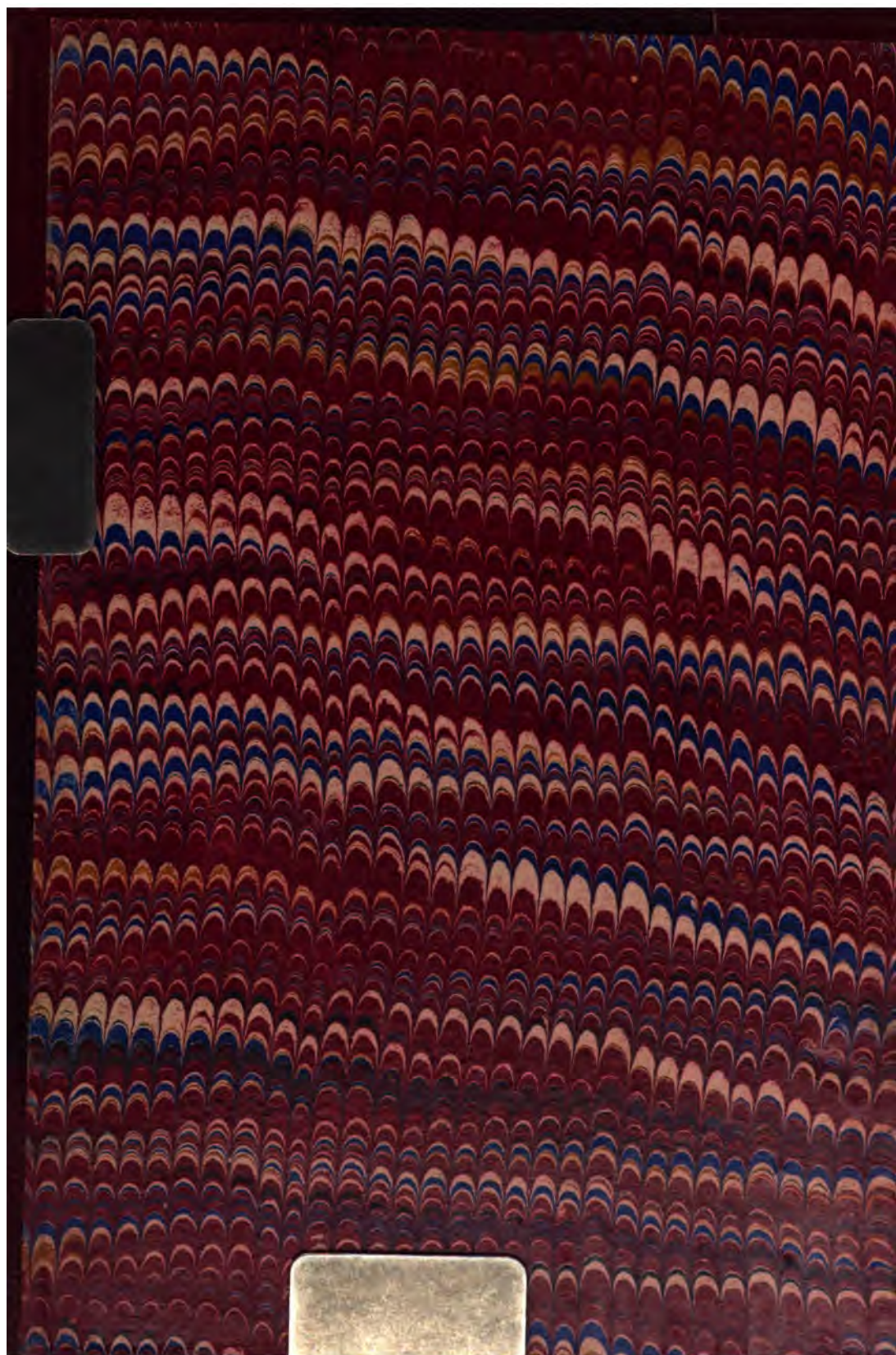




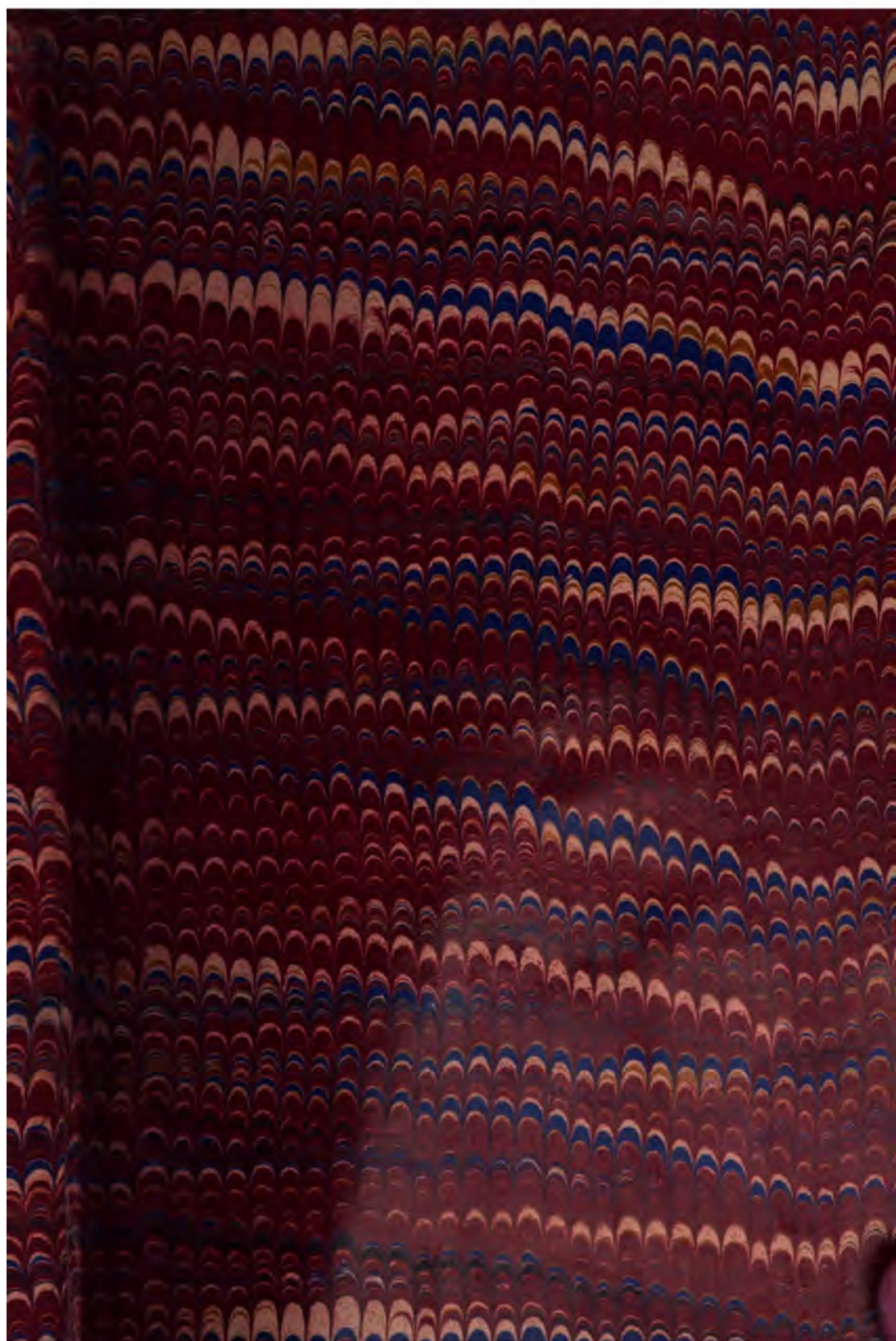














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Per. 1891 d. 67







JOHNS HOPKINS UNIVERSITY,

BALTIMORE.

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STUDIES

FROM THE

BIOLOGICAL LABORATORY,

Session 1877-78.

EDITED BY

H. NEWELL MARTIN, M. A., D. Sc.

PROFESSOR OF BIOLOGY.

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No. I.



BALTIMORE, MD.

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## To the Trustees of the Johns Hopkins University.

**GENTLEMEN:**

I beg leave to dedicate to you this little collection of first-fruits from the Biological Laboratory. It is to your liberal recognition of the fact that the duty of a University is not merely to diffuse but also to increase knowledge, that my fellow-workers and myself owe the opportunity of carrying on the researches described in the following pages.

The Zoölogical work accomplished at the Chesapeake Laboratory, maintained by the University during June and July last, will shortly appear in a separate volume.

Trusting that in future it may be my good fortune to present to you the record of greater achievements,

I have the Honour to Remain,

Your Obedient Servant,

H. NEWELL MARTIN.

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[*From the Journal of Physiology*, Vol. I. Nos. 2 and 3.]

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ERRATA.

In Dr. Martin's paper on "the normal respiratory movements of the frog, &c."

p. 144, line 10 from bottom, dele "e."

p. 144, line 5 from bottom, after "retraction" insert "e."

In Mr. Sewall's paper, on "the development and regeneration of the gastric glandular epithelium, &c."

p. 329, line 16 from top, instead of "latter" read "former."

1

**THE NORMAL RESPIRATORY MOVEMENTS OF THE  
FROG, AND THE INFLUENCE UPON ITS RE-  
SPIRATORY CENTRE OF STIMULATION OF THE  
OPTIC LOBES.** By H. NEWELL MARTIN, M.A., D.Sc.,  
*Prof. of Biology, Johns Hopkins University, Baltimore\*.* (Pl. VI.)

I.

THE frog has very frequently been employed by physiologists for investigations concerning the gaseous exchanges of the animal body under various conditions; but there exist remarkably few observations upon the nervous factors of its respiratory mechanism; so that while the mode of action of the respiratory centre of the mammal and the influence of various conditions upon its discharges are tolerably well known, there is an almost complete absence of corresponding knowledge with regard to the frog. It is known that its respiratory centre lies in the medulla oblongata (according to Flourens<sup>†</sup> well forward opposite the posterior border of the cerebellum), and that parts of the brain in front of this can be removed without causing cessation of the respiratory movements; but beyond this we know almost nothing. v. Helmholtz<sup>‡</sup> observed that stimulation of the central end of the pneumogastric nerve in the frog brings about cessation of the respiratory movements in that stage which is characterised by retraction of the throat. Heinemann<sup>§</sup> found certain respiratory disturbances to follow section of the pneumogastric trunks, but ascribes them to paralysis of muscles of the glottis. Goltz<sup>¶</sup> has shewn that if a frog's back be struck tolerably smartly against some solid object its respiratory movements cease for some time; and also that by irritation of the intestines its respiration is inhibited in the phase during which the external nares are closed, while stimulation of the skin causes cessation of the respiratory movements with the nares open. Von Wittich<sup>\*\*</sup> cites experiments on frogs tending to shew that the respiratory centre in them is reflex in character, apnoea occurring after the skin of the

\* The first part of this paper was read before the Maryland Academy of Sciences, Feb. 18, 1878; the second part before the Johns Hopkins University Scientific Association, March 6, 1878.

† See List of Authorities, p. 151.



animal is removed, even when the blood is extremely venous: he also states that if the lungs be extirpated and the animals therefore limited to cutaneous respiration they do not exhibit dyspnoea, but all respiratory movements cease and are only called forth by peripheral stimulation: the apnoea he also states to follow section of the vagi. Schiff<sup>28</sup> states that very strong induction currents applied to various regions of the body of the frog cause cessation of the respiratory movements in the expiratory state, with the throat relaxed. Strong stimulation in the coccygeal region however causes merely cessation of the movements of the nares, while the throat movements persist but with a slower rhythm. Rosenthal<sup>2</sup> ascribes these results to unipolar actions on the vagus, &c. This short list of observations, in some cases merely casual, on the influence of extrinsic influences upon the functional activity of the respiratory centre in this animal includes all that I have succeeded in finding.

The cause of this general neglect I imagine lies mainly in the apparent complexity of the respiratory movements in the frog which makes it somewhat difficult to arrive at a clear working idea as to the normal mode of action of its respiratory centre. *A priori* the frog would seem extremely well adapted for observations of the kind in question. In the mammal the results of experiment upon influences inhibiting or otherwise affecting the respiratory centre are almost invariably complicated by simultaneous changes in the blood gases (due to interference with the normal respiratory rhythm) altering the stimulus acting upon the centre; or if artificial respiration is employed to overcome this it introduces new complications through the afferent impulses excited in the vagi, by the expansion and contraction of the lungs, influencing the respiratory centre (Breuer<sup>29</sup>). In the frog we might expect to be largely freed from such secondary effects. Carrying on, as it does, a large proportion of its respiration through the skin, which, at least in winter frogs, seems perfectly sufficient to keep the blood in a state of normal aeration for a long time, the oxygenation of its blood is much less dependent upon the normal performance of the respiratory movements. In the winter frog, in fact, nature carries on an artificial respiration for us, which has the advantage of being independent of extension or collapse of the lungs. Moreover, its tissues in general, and in this we may expect the respiratory centre to share, shew a much slighter sensitiveness to deficiency of oxygen than do those of the warm-blooded animal. Wishing to make some experiments upon the influence of parts of the brain in front of the medulla oblongata upon

the force and frequency of the discharges of the respiratory centre, I turned my attention in the first place to the frog; influenced partly by the above considerations and partly by the ease with which operations upon its encephalon can be performed, and the rapidity and completeness with which the animal recovers from them.

It was in the first place essential to obtain a definite knowledge of the normal method of the frog's respiration as a preliminary step towards a knowledge of the working of its respiratory centre. This was the more important on account of the double character of the frog's respiratory movements, in some of which, as is well known, only throat muscles are concerned, while others are characterised by co-operation of muscles of the trunk; a doubleness which made it possible that the mode of action of its respiratory centre was essentially different from that of the mammal. When I turned to the literature of the subject I found that the statements on the subject were either too vague to be of use, in such treatises as that of Milne-Edwards<sup>10</sup> no notice being taken of the two kinds of respiratory movements; or that the statements made in the more detailed accounts of various observers differed in essential points. It became therefore necessary to investigate the matter afresh, and if possible by improved methods.

The physiologists of the end of the 17th and of the 18th centuries did not fail to observe that there was an essential difference in the mode of respiration of the frog and of the mammal; what chiefly attracted their notice being the fact that while the lungs of the latter collapsed when the chest was opened, those of the frog remained dilated, or if at first collapsed could be subsequently distended by the animal. Swammerdam<sup>7</sup>, Malpighi<sup>8</sup>, Morgagni<sup>9</sup>, and Laurenti<sup>10</sup> all refer to one or other of these facts, and give essentially correct accounts of the respiratory mechanism. On the other hand, Brémond<sup>11</sup> and Blumenbach<sup>12</sup> ascribed this faculty of remaining distended when exposed to the air to some specific power inherent in the frog's lung. Brémond's account is curious, for he admits in one part of his treatise that Malpighi's account of the mode of respiration in the frog is correct, but fails to see how this can account for the distension of the lungs when the visceral cavity is opened. He therefore concludes that fleshy fibres, which he has seen in the dried lung, are the cause of its dilatation during life, and points out that "*ces fibres doivent avoir beaucoup de force dans ce viscère et servir également quoique d'une façon différente pour sa contraction et sa dilatation*;" a sort of anticipation of the "active dilatation" of muscular fibres spoken of by

some physiologists as occurring in arterial walls. The first detailed account of the mode of respiration of the frog was however given by Townson<sup>13</sup> in 1794: his account far excels in minuteness of description those of any of his predecessors, and is substantially correct in all points. He describes not only the mechanism by which air is driven into the lungs, but also the mode of its expulsion by the contractility of the distended lungs, and the co-operation of the muscles of the wall of the visceral cavity. He pointed out that all the throat movements were not alike, but that some of them differed in character from the rest, and were alone accompanied with closure of the external nares and contraction of the flank muscles. He also described and figured the muscles of the throat, and first shewed that if the mouth of a frog was kept open it could not send air into its lungs, an experiment which Milne-Edwards erroneously states to have been first performed by Herhold<sup>14</sup>. The views put forward by Townson have been since generally accepted, but Rudolphi<sup>15</sup>, and subsequently Haro<sup>16</sup>, gave an essentially different account of the respiratory mechanism of the frog, by which it is assimilated to the mode of respiration in birds; the throat movements according to Haro being a mere freak of nature intended to conceal the actual process. The latter he states to consist in a compression of the lungs by an indrawing of the posterior end of the sternum due to contraction of the sternohyoid muscles which are attached to it. By this contraction air is driven out of the lungs, and so expiration is brought about: when the muscles relax the xiphoid cartilage by its elasticity returns to its former position, and dilates the cavity in which the lungs lie; consequently air enters and expands them. Inspiration is also in a subsidiary degree dependent upon the ascent of the hyoid carrying with it the glottis and trachea: by this the anterior parts of the lung sacs are expanded so that air passes into them. The experiments by which he attempts to prove the correctness of his statements are however of a highly inconclusive character, amounting to little more than the observation that the respiratory movements continue when an opening is made in the floor of the frog's mouth, a continuance which he considers to prove that actual functional respiration was performed: air enters the lungs since expiration occurs as ordinarily, "*ce que prouvent les contractions de l'abdomen et des flancs.*" This is of course equivalent to saying that a mammal can breathe when its thorax is opened, because one sees the respiratory movements of the ribs and diaphragm to continue. The further proofs offered by him are of little more cogency, consisting in the fact that a frog treated as



above and with its visceral cavity opened may live several days; and that when the animal's mouth was held open the currents of air could be felt passing out from the glottis when the sterno-hyoid muscles contracted. By this latter statement, which at first sight might seem to prove the existence of some such respiratory mechanism as he describes, it seems clear that he only refers to the expulsion of air which might already be contained in the lungs when the observation commenced, for he says the best way of seeing it is to hold the animal under water and see the bubbles rise: under such circumstances there could be no question of an entry of fresh air into the lungs. Direct observation of the lungs, when the floor of the animal's mouth was removed, shewed him a "sensible" dilatation of the anterior parts of those sacs during the period at which he supposed inspiration to be taking place, while their posterior extremities shewed no dilatation. He speaks of the changes in the diameters of the chest wall, which he considers the essential part of the respiratory mechanism, as not being sensible: such feeble movements as these are, in his opinion, the actual respiratory movements of the frog; the obvious movements of throat and flank being of little or no significance. Panizza", incited by Haro's statements, undertook an experimental investigation of the question, and while confirming Haro as regards Chelonians, proved very clearly that his account of the respiratory mechanism of the frog was erroneous, the agents which he regarded as the sole ones being at the most quite subsidiary. Panizza removed the skin from behind the fore limbs of the frog, and was thus able to watch the lungs through the thin muscular wall of the visceral cavity. He was then able to see that they dilated and contracted considerably when the throat and nares were left intact; but that when the floor of the mouth was removed, as described by Haro, or when the outer border of the nostril was cut away, so as to prevent its closure, then the lungs soon collapsed; and during the inspiratory movements dilated extremely little, and only at their anterior part. When a tympanic membrane was removed the lungs similarly collapsed; but if the opening were closed by the finger they were soon dilated, and the dilatations could be seen to occur at the moments of retraction of the throat. All these phenomena were quite irreconcilable with Haro's view of the respiratory mechanism, and proved conclusively that the older view was in the main the correct one, even if some such action as Haro imagined occurred in extreme dyspnoea. Panizza also shewed that if a frog's glottis were hermetically closed the animal could live in a room at

7° to 8° for twenty-one days; so that the fact that Haro's frogs lived some five or six days did not in the least prove the occurrence in them of any active lung respiration. The insufficiency of continued vitality as a proof of pulmonary respiration in the frog had also been shewn previously by the experiments of W. Edwards<sup>30</sup>. Panizza also observed, as Swammerdam<sup>18</sup> and Townson<sup>18</sup> had previously done, that the frog sometimes diminishes its mouth cavity in order to drive air into its lungs, not only by retracting the throat but by drawing in the eyeballs. Heinemann<sup>8</sup> seems to have been the next to take up the question with thoroughness, and his article is extremely good. Accepting Townson's account in the main he supplements it in various points, the more important of which are as follows. The contraction of the flank muscles (which, as Townson pointed out, only accompanies the more powerful throat movements) follows the descent of the throat, occurring at the moment when it again begins to ascend. With the smaller throat movements the abdominal walls shew slight variations which are brought about by changes of place in the hyoid. The glottis is closed during descent of the hyoid; immediately after the ascent commences it opens widely, to close again when the throat reaches its highest position. The exact discrimination is however not easy, and can only be attained after numerous experiments, since decapitated frogs, on which these observations were made, commonly breathe differently from normal frogs. If the brain be almost completely removed (with the fore part of the head so as to expose the glottis) the frog sits for some time, often many minutes, without movements of the throat: when these recommence they are usually all of the more energetic type, and then always accompanied with opening of the entrance to the larynx. In a few cases however the feebler movements are seen, and during these the larynx remains closed. He points out the great differences seen in frogs as to the ratio in number of the more feeble to the more powerful respirations in a given time: the latter, which alone he calls respirations, may occur only once in 2—3 minutes, but may on the other hand rise from 66—104 in one minute, the latter when both vagi are cut. The more feeble throat movements he thinks may be explained by supposing that the impulses starting from the medulla oblongata must have a certain strength in order to call forth the whole series of respiratory movements. The throat muscles might be set in work without simultaneous stimulation of the glottidean muscles; which latter first occurs when the stimulation at the centre has reached a cer-

tain height. Expulsion of air from the lungs immediately precedes its entry, so that the movements causing the two are almost continuous. As will be seen presently, my own observations have led me to differ from Heinemann as to the relationship in time of the throat movements and the contraction of the flank muscles: simultaneous tracings from throat and flank shewing that the flank contracts at the end of the descent of the throat, and not at the beginning of the ascent. If this be so, he is also slightly in error as to the time of opening of the *aditus laryngis*, which he says is difficult of discrimination; the opening for the expulsion of air must first occur, when the lungs are compressed by the flank muscles, and therefore before the ascent of the throat.

Bert<sup>19</sup>, so far as I have been able to find, was the first to apply the graphic method to the study of the respiratory movements of the frog, but a critical examination of his experiments shews that they are not entirely satisfactory. He makes no reference to the distinction between the two types of respiratory movements, so that it is not always possible to say whether his tracings are those of respiratory movements accompanied by closure of the nares and contraction of the flank-muscles, or whether they are derived from respiratory movements not so accompanied. In his figure 1 for instance, in the *Journ. de l'Anat. et Phys.* (Fig. 52 in his book<sup>20</sup>) all the respiratory curves are alike, and must all be due to the same kind of respirations. The tracing was obtained by placing a tight-fitting bag, connected with a Marey's tambour, over the frog's nose, and so recording the passage of air into or out of the nostrils. Now if a frog be placed in a small beaker (to exclude external air currents), and when it is breathing quietly a small flock of cotton-wool be held above and a little to the outside of one nostril, the wool will be seen to be blown about by the air expelled from the nares during each kind of respiratory movements. But there is no comparison between the violence with which it is blown in the two cases, its movements being much greater when the flanks contract. From this obvious difference in the force with which the air is expelled it is impossible to conceive that the tracings derived from the tambour should be the same in the two cases, and we have to decide which of the two alone is depicted in Bert's figure. From the letterpress it is clear that the respirations accompanied by flank-contraction are those from which the tracing was taken; and such an uninterrupted series of them only occurs in dyspnoic or otherwise abnormal conditions. Bert's statement, originally made by Panizza, that normally the frog when

driving air into its lungs does not completely close its nares either externally or internally, but merely narrows them, is probably correct, though his further statement that the animal can under no circumstances do so, seems inconsistent with one of Panizza's experiments. The latter observer found that if the head of a living frog was held immersed in a solution of potassic ferrocyanide for some minutes and then removed, the mouth carefully opened and solution of ferric chloride applied to the internal nares, no blue colour was developed. Another of Bert's experiments devised to shew that Haro's views were incorrect is unsatisfactory. He put a tightly-fitting cannula containing liquid (or as described in his book, connected with a recording tambour) in the glottis of a frog, and saw no changes of level in the fluid or movements of the lever: this he ascribes to the mouth being held open by the cannula or to the air being, in other cases, able to pass out through the opening in the pharynx made for the introduction of the instrument, and he concludes that no such inspiratory mechanism as that described by Haro could in this case be active. But here he can only have been dealing with the more feeble respiratory movements, the flank respiratory movements being probably inhibited by the sensory stimulus caused by the tube in the glottis: for if the flank muscles had contracted, thereby compressing the lungs, they must have caused changes in the level of the liquid or in the lever. He states, however, that when the lungs are previously extremely collapsed slight changes in the level do occur, from which he concludes that the mode of respiration described by Haro has, in these conditions, a certain amount of efficacy. Similar objections apply to Bert's second figure (Fig. 53 in his book): this figure gives simultaneous tracings of the changes of pressure in tambours connected by cannulæ, one with a nostril and the other with the cavity of one lung. Here again all the curves are similar, with one slight exception, to which he makes no reference, and which is probably accidental. The changes of pressure in the lungs are, moreover, so trivial, compared with those in the mouth, as to make it almost certain that the glottis was closed during the whole time; the respiratory movements being all of the feebler type: and such slight variations of lung-pressure as did occur, being due to a stretching of the anterior part of the lung by the ascent of the hyoid, in the manner pointed out by Heinemann. Although Bert's experiments are not conclusive, his statements as to the mode of respiration of the frog are, nevertheless, in the main, correct, with the exception of his omission to point out the two degrees of respiratory movements which normally occur. In one



point he is, I think, more correct than Heinemann, viz., in stating that air passes out of the lungs immediately before the commencement of the retraction of the throat. In his table shewing the conditions of nares, throat, glottis, and lungs, during the phases of a respiratory period, it is significant that no mention is made of the abdominal walls; an omission which goes to confirm the suspicion that his plan of introducing cannulæ into the glottis inhibits the more powerful expiratory discharges.

Burdon Sanderson<sup>20</sup> has also described the respiratory mechanism of the frog, and given a figure of the curves—obtained from a tambour connected with a cannula placed in one nostril. The curves obtained by him shew marked differences corresponding with the feebler and more powerful respiratory movements, which makes it quite clear that Bert was dealing with only one kind. Sanderson's description of his figure does not, however, so far as I can make out, agree with the figure itself: and his statement that the entry of air into the lungs immediately precedes its expulsion, so that it remains a very short time in those cavities, is at variance with the statements of all other observers and with the results of my own experiments. He states that the contraction of the flank muscles immediately follows closure of the nares and occurs while the hyoid is still drawn upwards. He makes this statement, partly on the ground of direct simultaneous observation of the movements, which is however very difficult to carry out, and partly on the study of the intra-pulmonic pressure: and it is this latter which I think has misled him. He takes the sudden rise of intra-pulmonic pressure to indicate the entry of air into the lungs: it is, I believe, rather due to the compression of the lungs by the flank muscles, the glottis being passively closed. Immediately afterwards, the glottis is actively held open, and the flank muscles having relaxed, air enters the lungs under a less pressure than that at which it was driven out of them. Townson's observation that one lung only may be collapsed in correspondence with a contraction of the muscles of only one flank seems to prove that the glottis is not actively held open during contraction of the flank muscles, for if such were the case, both the distended lungs would almost certainly collapse, the abdominal walls being so flexible.

Whatever be the explanation, simultaneous tracings of throat and flank movements shew that the flank muscles contract, expelling air from the lungs, when the throat is still protruded by descent of the hyoid: that the lungs are distended immediately after this contraction;

and the air remains in them a considerable time, viz. until the next flank contraction.

In giving an account of the statements of Heinemann, Bert, and Sanderson, I have anticipated somewhat in criticising them; it now remains for me to give the details of the experiments upon which the criticisms which I have ventured to make are founded. The preceding abstract of the history of the investigation of the respiratory mechanism of the frog shews that while tolerable unanimity upon its broad features has been reached there is still a great discrepancy upon points of detail, and points of which it was essential for my purpose that I should have a clear idea. This was especially the case as regards the time relationships of the throat and flank movements. While it was pretty definitely settled that the minor throat movements sent no air into the lungs as the larger did, and that the flank movements and those of the nares accompanied only the larger, there was a wide difference of opinion as to when these flank movements occurred. Heinemann says they take place at the commencement of the throat retraction: Sanderson, if I interpret correctly his phrase, "while the hyoid is still drawn upwards," at the end of the period of throat retraction: and Bert, at the end of the phase of throat protrusion. If the statements of Heinemann or Sanderson were correct, the discrimination of inspiratory and expiratory discharges from the respiratory centre of the frog became very difficult; for the throat retraction, essentially an inspiratory movement, would coincide in part with the essentially expiratory movement of contraction of the muscles of the abdominal wall. The discharges of the inspiratory and expiratory centres would, in other words, overlap: and for my purpose it was necessary to discriminate them. If it did turn out that the discharges of the two centres could, in part, coincide, that fact in itself would be of great importance. Some of the movements in question being extremely rapid, and the time differences involved extremely small, it was essential to record them simultaneously, and extremely desirable to do so with the frog breathing as much as possible in a natural manner, and if possible in its normal position. For the latter reason I rejected the employment of tambours which necessitated interference with the movements of the nares, and the opening of a lung, and which had besides led to discrepant results in the hands of Bert and Sanderson, and attempted to register the movements of throat and flank directly. At first I did not hope to be able to record in this way the respiratory movements of

the unconfined and uninjured frog, and I therefore employed animals whose cerebral hemispheres had been removed. I have, however, recently succeeded in getting tracings of the throat movements in the uninjured frog sitting free in its natural position, and the complete similarity even in the small details of the two tracings leaves no room for doubt that the whole respiratory movements in the frog with and in that without the cerebral hemispheres are the same: a conclusion which direct observation fully confirms.

The throat tracings were obtained by means of a light lever, which was almost equipoised so as to exert only enough pressure upon the throat to ensure that it followed the movements of the latter. The writing-point of this lever, made of aluminum foil, traced upon the smoked surface of an ordinary revolving cylinder. The lever itself did not rest directly upon the throat of the frog: I found this caused too much irritation so that the animal either jumped away or pushed off the lever before a tracing could be taken; and it had the further disadvantage that the ramus of the jaw sometimes prevented the lever from following the throat when the latter was in extreme retraction. To obviate these disadvantages I fastened a small piece of cork to the lever near its fulcrum; into this a pin was stuck in a horizontal position; the free end of this pin carried another piece of cork and into this was inserted the point of another pin which was placed in a vertical position, and the head of which pressed gently on the frog's throat; and so any movement of the latter was transmitted to the lever. If the pin-head be very gently brought in contact with the throat (which in my apparatus was done by a rack and pinion movement) an uninjured frog will frequently bear the contact, and give a tracing for more than half a minute\*. Frogs whose cerebral hemispheres are removed are frequently very troublesome at first, jumping away the moment the apparatus touches them; but when once they have borne the lever for a short time they usually give little trouble afterwards, seeming to get used to it; and when they do move they commonly either merely push the lever away with one foot or turn aside from it instead of jumping away. I have found it a great help to make use of Tarchanow's<sup>28</sup> observation that compression of the abdominal cavity lowers the reflex irritability of the frog: if the animal

\* My tracings from the uninjured frog were obtained in midwinter, and the frogs had probably not quite their normal irritability. They had however been for several days in a room at a temperature of from 15° to 18° C. and were vigorous and lively, jumping with great activity when irritated.

be seized between the finger and thumb just in front of the urostyle and gently squeezed it will usually stay where it is placed, and the lever can be adjusted before it again gets irritable: this pressure nearly always leads to complete cessation of the respiratory movements for some seconds after it is removed, and this also facilitates the adjustment of the lever. When the animal wakes up again and begins to breathe, it commonly takes no notice of the lever for many minutes. None of my tracings was taken until at least a minute had elapsed after the resumption of the respiratory movements, so as to eliminate any possible effects of the previous cessation, or pressure.

The frogs which I have employed were nearly all partly-grown specimens of *Rana lentiginosus*, about the size of full-grown specimens of *Rana temporaria*. The animal was placed on a sheet of lead which formed a slightly inclined platform in a trough filled with water. The highest edge of this platform was in contact with one end of the trough and level with it: over this end of the trough the throat of the animal projected and had the pin-head of the lever apparatus in contact with it; while in consequence of the slope of the platform the posterior limbs and body of the animal were partly immersed in the water. A thermometer in the latter was used to indicate if any considerable changes in the temperature of the room occurred during the more prolonged experiments; as the force and frequency of the frog's respiratory movements are subject to great changes with alterations of temperature; wherever temperatures are given in the details of the following experiments they are those indicated by this thermometer.

Figures 2 and 3, Plate VI, give tracings of the throat movements of the uninjured frog as obtained in the manner just stated: Figure 4 is a similar tracing from the frog with its cerebral hemispheres removed; and it will be seen that it is identical in character with those obtained from the normal animal. In these tracings, ascents of the curve indicate throat retractions, and descents, throat protrusions; and it will be seen that at somewhat irregular periods there occur more marked protrusions followed by more powerful and sudden retractions: these are the throat signs of those respirations which are accompanied with the closure of the external nares and the active contractions of the flank muscles: and the first point was to decide in what relation these three events stood to one another. As to the contraction of the nares there can be no doubt that it accompanies the more powerful throat retractions. There is considerable difficulty in observing simultaneously the nares and the throat, but it is much more easy to watch



at the same time the lever and the nares, and this indicates the relationship just stated; which is moreover that universally accepted, the occlusion of the nares simultaneously with the more powerful protrusions of the throat being *a priori* extremely improbable. For it is agreed by all observers since Haro that it is during the marked throat retractions that air is driven into the lungs of the frog. The main point then which I had to determine, was the relationship in time of the various phases of the throat movements to the contractions of the flank muscles; a point on which Heinemann, Bert, and Sanderson differed. With this object I placed a second rectangular lever, so that its short vertical limb was in contact with the frog's flank, while its longer horizontal limb wrote on the revolving cylinder, immediately over the throat lever. The tracings thus obtained shew conclusively that the flank contraction immediately precedes in each case the powerful throat retraction, being in fact contemporaneous with the powerful throat protrusion; or rather with that part of it which is characterised in Figures 2, 3 and 4, at the points *a*, by a more sudden descent of the lever. Figures 6 and 7 represent such simultaneous tracings, and it will be seen that the flank contractions indicated by the descents *b* in the upper tracing of each figure immediately precede the powerful throat retractions *c*, by which air is driven into the lungs. The tracings further shew that from this time the lungs remain filled, as indicated by the continued protrusion of the flanks, until the next active flank contraction, so that the air, far from remaining a brief time in them, stays there for a considerable period. Otherwise we should have collapse of the lungs attended with protrusion of the flexible walls of the cavity containing them. The air therefore enters the lungs immediately after its expulsion by the flank muscles, and not immediately before it, as stated by Sanderson. The minor curves, *d*, described by the flank lever during the minor throat movements, have nothing to do with an entry of air into the lungs or its exit from them, as I shall shew presently. The break in the ascent of the lever at *f* while air is entering the lungs is due to its inertia. Heinemann also must be wrong in stating that the glottis only opens when the throat commences one of its more powerful retractions. It must open just before this when the flank muscles contract; but probably this opening is passive and inconspicuous as compared with the active inspiratory dilatation of the glottis which succeeds it.

The tracings from which Figures 6 and 7 are copied were, it is true,

obtained from frogs whose cerebral hemispheres were removed: but the exact agreement of the throat movements in such frogs with those of uninjured frogs makes it impossible to believe that their normal respiratory modus has been altered. Certainly not to the extent of reversing the usual time relationships of the throat and flank movements.

In the tracings it will be noticed that the ratio of the number of the major to the minor throat movements is subject to considerable variation. In Fig. 6, after a period without major throat movements or flank contractions, a number occur close together; and the inspiratory effect on the whole overbalances the expiratory, the lungs becoming more and more distended, as shewn by the gradual ascent of the upper tracing. In Fig. 7 we find a powerful emptying of the lungs following one of these periods of dilatation and then no active flank contraction again for a considerable time. Such a grouping of major throat movements as is shewn in Fig. 6 is unusual. In Fig. 5, taken from an uninjured frog of a different species (which I have not been able to determine), the rhythm is very regular, and the major throat movements are unusually numerous; whether this is an individual or a specific characteristic I do not know; it probably represents a somewhat dyspnoëic condition.

I have gone with this detail into the account of the frog's respiratory movements because those of the throat being by far the most easily recorded it is of great advantage to be able, if possible, to interpret tracings from the throat so as to be able to read from them what is happening at the same time in nares, flanks, and lungs. If I am right in the conclusions above stated, it will be seen that this is not only possible but easy. In Figs. 2, 3, 4 and 5 the smaller curves, *g*, will indicate throat movements unaccompanied by closure of the nares or by any change in the volume of air in the lungs, but simply renewing the air in the mouth. The more marked protrusions of the throat, *e*, on the other hand, will be accompanied towards their termination at the points marked *a* (usually differentiated by a sudden alteration in the curve, but sometimes appearing merely as a prolongation of the period of protrusion, as is well seen in Fig. 6) by active contraction of the flank muscles and the expulsion of air from the lungs. The powerful throat retraction following this is accompanied (at any rate in its earlier part) by more or less complete closure of the nares, the entry of air into the lungs, and passive protrusion of the flanks: from that time the lungs remain distended during the succeeding smaller throat movements until the next powerful throat pro-

trusion is accompanied again with flank retraction and expulsion of the air. To facilitate description I shall in future designate the respirations indicated by the smaller throat movements simply as "throat respirations:" and those accompanied with the change of air in the lungs as "flank respirations," though of course the throat shares in these latter also.

There were two other points on which it was desirable to obtain tracings. Heinemann has pointed out that if the frog's flank be very carefully watched, movements in it will be seen to take place even during the "throat" respirations. These he ascribes, as above stated, not to an entry or exit of air from the lungs or to active contractions of the flank muscles, but to an increase of the pleuro-peritoneal cavity in its antero-posterior diameter by the ascent of the hyoidean apparatus, which increase is compensated for by a diminution in the transverse diameter; the contents of the cavity remaining the same and no air entering or leaving the lungs. If this be so, the throat retracting and pulling the lungs up out of the visceral cavity, without at the same time dilating them, as happens in the throat retraction period of the "flank" respirations, then the flanks ought to shrink when the throat is retracted in these "throat" respirations. Simultaneous tracings of throat and flank during the "throat" respirations (Fig. 1) shew this to be the case. The upper tracing is from the flank; ascents in it represent flank protrusions: the lower tracing is from the throat; descents in it, as before, indicate throat protrusions. It will be seen that when the throat protrudes the flank protrudes, and *vice versa*; exactly the reverse of what occurs in the "flank" respirations. Heinemann's explanation of these slight passive flank movements is therefore the correct one. In this way the minor flank movements *d*, in Figures 6 and 7, above referred to, are produced. These movements must not however be confounded with feebly active flank movements which occur in certain dyspnoëic states with each throat movement. In such cases these active flank contractions are readily differentiated by their suddenness. It will be noticed in Figure 1, that a considerable series of "throat" respirations occurred, uninterrupted by any "flank" respiration: a phenomenon to which several observers have referred.

Finally it remained to be seen whether there was any fragment of truth in Haro's view of the frog's respiratory mechanism: Panizza, Bert and others thinking that possibly some such action as he describes may occur as a subsidiary phenomenon, at any rate in dyspnoëic

states. To investigate this I fastened an uninjured frog upon its back by strings passed round its limbs, and recorded by levers simultaneously the throat movements, and those, if any, of the posterior end of the sternum: in some cases the flank movements were also recorded. Such tracings shewed that at the time of active contraction of the flank muscles (expiration) the lower end of the sternum was slightly pulled inwards towards the vertebral column: on account of its elasticity, this end will no doubt tend to return to its position of equilibrium when the expiratory muscles relax, and so will passively tend to bring about an inspiration by dilating the chamber in which the lungs lie. The amount of this influence must however be very small; and it is open to doubt whether the inbending of the sternal end is not due rather to the contraction of the expiratory muscles in the abdominal wall than to the sterno-hyoids: especially as Heinemann could see no change in the position of the posterior end of the sternum, to be brought about by tetanising the latter muscles. In extreme dyspnœa these movements of the posterior end of the sternum become considerable.

Such being the normal respiratory movements of the frog, it became my next object to gain some idea if possible of the relationship in which the activity of the respiratory centre in this animal, as indicated by its respiratory movements, stood to that of the mammal, with its rhythmic inspiratory and expiratory discharges, innervating different groups of muscles. The muscles concerned are of course widely different in the two cases; but that in itself gives no reason to conclude that the fundamental working of the respiratory centres should not be the same in both. At first sight however the frog's respiration seems to be essentially different: it looks as if we had in it to deal with two distinct respiratory centres, each with its inspiratory and expiratory division, and concerned respectively with the "throat" and "flank respirations," which seem to have a rhythm quite independent of one another. The centre for the "flank respirations" would then be characterised by the greater violence of its discharges and their less frequency, as well as by their radiation to muscles not under the influence of the other centre. The fact, however, that under certain conditions, as dyspnœa and, as I shall shew presently, of excitement of the optic lobes, the flank and throat respirations gradually shade off into one another, is against the hypothesis of two totally independent centres; and I think the whole mechanism can be explained by the working



of a single inspiratory and a single expiratory centre, and without doing violence to what is known concerning the mode of action of those centres in the mammalia. That we have to do with only a single respiratory centre, with its inspiratory and expiratory divisions, is the view adopted by Heinemann, as quoted above. His attempt to account for the different amounts of the discharge in the two cases is however not satisfactory. He supposes that the stimulus acting upon the centre is at first only powerful enough to cause discharges which radiate to the throat muscles. These bring no air into the lungs; the stimulus to the centre therefore increases, and causes finally a discharge which radiates to more muscles, causing what I have called a "flank" respiration and renewing the air in the lungs. But if this were the whole matter then the throat respirations ought gradually to increase in strength and in the number of muscles concerned, until the last of the series passes into a "flank" respiration; and this my tracings shew is not the case. In the normally breathing frog there is no transition, but a sudden jump from one to the other.

If the brain and spinal cord of a frog be destroyed and the animal be then held in its natural position, it will be seen that the throat assumes the protruded state: so that this is its position of equilibrium in the normal posture of the animal; and that to which it will return when muscles which have removed it relax. This protrusion is sometimes not quite so great as that which corresponds to the position of the throat in the protrusion phases of the "throat respirations"; but in such cases it will be found that, as is commonly the case, the lungs have been entirely emptied of air during the operation; so that instead of being moderately distended as is their condition during the normal "throat respirations," they are now in a state of extreme collapse, indicated by the abnormal retraction of the abdominal walls. In this general retraction of the soft boundaries of the body cavity the throat shares somewhat; and moreover the larynx is probably pushed out somewhat, in normal conditions, by the distended lung, and this influence also is now entirely removed. In fact if in such cases the lungs be moderately distended by blowing air through the glottis, and the animal be then held again belly downwards, it will be seen that its throat assumes a position of protrusion quite as great as that which occurs in the "throat respirations." We may conclude that in these respirations we have only to do with discharges of an inspiratory centre, leading to contractions of the elevators of the hyoid apparatus; the throat protrusions being passive. These respirations will answer

closely to those which take place in the normal breathing of many mammals, where the expirations are passive; and in the frog, as in them, we shall have a more irritable or a more readily discharging inspiratory centre, and a less irritable or less readily discharging expiratory centre\*. If the stimulus to the inspiratory centre was gradually increasing while the series of throat movements went on, one of two things must happen: either the throat movements would increase gradually in extent, or they would more probably (the resistance to the discharge remaining the same) become more frequent; but they do neither. This can be seen in Figs. 2, 3 and 4, and especially well in Fig. 1, where we have a long series of uninterrupted throat movements, and shows how efficient in winter frogs the skin respiration is, serving to keep the stimulus to the respiratory centre constant for a long time. When the throat respirations have gone on for a time, which is subject to considerable variation, the stimulus which has meanwhile been acting also upon the expiratory centre finally attains a degree which arouses that centre to discharge; and the result is an active expiration with contraction of the flank muscles, and usually of the depressors of the hyoidean apparatus; as is shewn by that protrusion of the throat beyond its position of equilibrium which is indicated by the greater descent of the curves in Figures 2, 3, 4, and 5 at the points marked *a*. Sometimes however the discharge does not radiate to these muscles, and the activity of the expiratory centre then is only indicated on the tracings obtained from the throat by a prolongation of the period of protrusion. This is well seen in Figure 6 at *a*, and characterises certain frogs throughout. A more difficult question now arises: how to account for the greater contraction of the elevators of the hyoid in the immediately following inspiration, and the radiation of the discharge to the muscles of the nares and glottis, without calling in the aid of an inspiratory centre different from that which brings about the ordinary throat retractions; or without assuming some special stimulus acting upon the inspiratory centre at this moment, and corresponding to nothing which occurs in the mammal? The answer which I venture to propose to this question is the following. In the first place it will be noted on the tracings that the interval between this inspiration and that which preceded it is always longer than the interval which separates the inspirations of the "throat respirations" from one another; the activity of the expiratory centre has apparently inhibited the inspiratory centre, or increased the resistance to its discharge; and

\* See Lockenberg<sup>22</sup>.

the normal stimulus thus acting longer upon the centre would be apt to cause an unusually powerful discharge when the inhibiting influence or the increased resistance was removed (Rosenthal<sup>1</sup>, p. 246). That the unusually excited centre should not only stimulate to more vigorous contraction the muscles which it usually affects, but should radiate impulses to others, is only in accordance with what we know of its activity in other cases. The experiments of Breuer<sup>22</sup>, confirmed by Lockenberg<sup>23</sup>, have moreover shewn that contraction of the lungs, acting through certain fibres of the vagus, facilitates inspiratory discharges in the mammal; and if we suppose this to be true of the frog also, we should expect a more vigorous inspiratory discharge to follow the active expiration with its attendant collapse of the lungs; this latter probable influence needs however experimental confirmation for the frog; and this I have not yet had the opportunity to undertake. But in one or both of the above agencies we have, I believe, a sufficient explanation of the more powerful inspiratory discharges of the "flank respirations."

It will be seen that in the above I have spoken of the centre which innervates the elevators of the hyoid as an inspiratory centre throughout, although the majority of its discharges lead to no entry of air into the lungs. If however this centre is, as I believe, the same as that which from time to time does bring about the propulsion of air into the lungs, the only difference being that in some cases its discharge radiates more widely than in others, and affects new muscles, it is undesirable to give it two names; it is the inspiratory centre, although sometimes its activity drives air out of the mouth, not into the lungs but to the exterior of the body. This frequent renewal of the air in the mouth cavity will lead to its being nearly as pure as the external air; when the active expiration takes place and the lungs are emptied, some of this pure air must be left in the mouth, and, in the immediately succeeding inspiration, will be sent into the lungs as a sort of "tidal air" with some of the air just expelled from them, which will correspond to the "stationary air" of the mammal. The considerable time that the inspired air commonly remains in the lungs is probably correlated with the simplicity of their structure. Almost simple sacs, little impediment is in them opposed to the renewal by gaseous diffusion of the layer of air in contact with their inner surfaces; and so there is not the need for that promotion of the mixture of the layers of air by frequent mechanical movements which is requisite in the complexly subdivided mammalian lung. Moreover the large cavity of the lungs in comparison to the

surface in which the capillaries lie renders the frequent renewal of the air unnecessary.

The fact that many respiratory movements in the adult frog have no immediate connection with the renewal of the air in the lungs has a special interest, for it seems probable that it is a physiological remnant of the frog's larval and ancestral mode of respiration. The tadpole possesses for a time, and ancestors of the frog possessed probably throughout their whole life, both lungs and gills; while immersed, water must be driven over the surface of the gills by contractions of the mouth cavity; and it is of course important that this water should not at the same time be sent into the lungs, so the dilators of the glottis must not be stimulated. This renewal of the water will then be due to movements answering to those which I have called "throat respirations." Finally when the nares come to open into the mouth and the gill openings close up, these movements remain; but they now drive air out through the nostrils instead of water through the gill chambers, and so without any sudden change in its nerve centres, which otherwise would seem unavoidable, the frog becomes an entirely air-breathing animal, except such respiration as may be carried on by its skin while under water. While the gill openings remain, if the animal is to inflate its lungs with air, there must be a means of closing these openings; and Martin-Saint-Ange<sup>28</sup> has shewn that a very complete apparatus for such closure exists in the tadpole of the salamander. In the frog's tadpole this apparatus is less complete, but from the more enclosed condition of the gills he thinks it is efficient in this case also. If the common statement be correct, that frog tadpoles frequently require to come to the surface to breathe in the later stages of their development (but while still possessing functional gills), the apparatus for closing the gill clefts, or the gill sac, must in fact be efficient. Otherwise I should be inclined from Martin-Saint-Ange's description to doubt the functional utility of the closing apparatus in these tadpoles.

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## II.

HAVING in the manner above described attained at least a working hypothesis as to the normal mode of action of the respiratory centre in the frog, I was able to set to work with more definiteness on the investigations of influences altering its functional activity; and first I turned my attention to the influence of stimulation of the *corpora bigemina*, the so-called "optic lobes." Even in the mammal very little is known concerning the influence of parts of the brain in front of the *medulla oblongata* upon the respiratory rhythm. It is known of course that respiratory movements persist in some fashion when all these parts are removed: and that the will, whose manifestations are correlated with the integrity of some of them, is capable, within limits, of governing the discharges of the respiratory centre. The influence of sensory stimuli is also known to act through the mediation of the cerebral hemispheres; and according to Rosenthal<sup>1</sup> the inspiration-inhibiting influence of the recurrent laryngeal nerve, first demonstrated by Burkhart<sup>2</sup>, is exerted similarly. Danilewsky<sup>3</sup> states that removal of the cerebral hemispheres in the mammal makes the respirations slower but more energetic, the inspiratory movements becoming more powerful. During the last few years also various observers have found alteration in the respiratory rhythm to accompany electrical stimulation of various localities in the anterior portions of the brain. Thus Danilewsky<sup>3</sup> found the respiratory movements of dogs and cats slowed with an initial deeper inspiration by stimulation of the cauda of the corpus striatum. Stimulation of medium strength brought about a deep inspiration, followed by a slow expiration, and then complete cessation of the respiratory movements, which often lasted longer than the stimulation. He saw the same results follow stimulation of the "facial centre," though he is in doubt whether in the latter case the result was not due to escape of the current to deeper parts: he also found that stimulation of the corpus striatum caused cessation of a quickening of the respiratory movements brought about by stimulation of the tibial nerve. Ferrier<sup>4</sup> states, erroneously, that Dani-

lewsky had found electrical irritation of the interior of the *corpora quadrigemina* to cause a deep inspiration, followed by prolonged and powerful expiratory efforts, and had in this anticipated results obtained by Lauder Brunton and himself. From this I conclude that Brunton and Ferrier have found these results to follow stimulation of the *corpora quadrigemina*. Balogh<sup>4</sup> found that stimulation of certain convolutions and of the posterior part of the corpus striatum caused more frequent respirations; while stimulation of parts of the suprasylvian fold caused cessation of the movements.

As already stated, Flourens has shewn that the respiratory centre of the frog lies well forward in the medulla opposite the posterior edge of the small cerebellum. The comparison of tracings such as that represented in Fig. 4, of which I have many, with the tracings taken similarly from the normal frog, shews that the removal of cerebral hemispheres and optic thalami in this animal produces no alteration in the respiratory movements: unless perhaps a slight tendency to greater regularity in the ratio of "throat" to "flank" respirations in a given time. When the optic lobes are removed, I am inclined to believe that the respiration is affected, but I have never been able to satisfy myself that animals upon which this latter operation had been performed recovered a physiological state sufficient to give a fair basis for comparison. Whether this be so or not, I have almost invariably found that such frogs breathe much less often than normal frogs, or those with *corpora bigemina* intact. The observations of Setschenow<sup>5</sup> and others tending to demonstrate the existence in the *corpora bigemina* of a centre inhibiting the reflex actions of the spinal cord made it especially desirable to see if there were not also in them centres influencing the centre for the respiratory movements, which if not reflex in its normal working is at least largely subject to reflex control.

The general method of operating in order to remove the fore parts of the brain of the frog is too well known to need a detailed description here. After dividing the skin in the middle line, I have always removed a piece of the skull with a small trephine applied in a lozenge-shaped area which is seen to be bounded on the sides by four small vessels. The posterior edge of the opening thus made extends back to about opposite the posterior margin of the cerebral hemispheres, and the aperture was enlarged with scissors until the front edges of the optic lobes came into view. These were carefully and completely separated by a cataract knife from the parts of the brain in front of them, and

the latter were removed from the cranial cavity; the incision in the skull being usually carried forwards to facilitate this removal. The edges of the skin were then brought carefully in contact, without sutures, and the animal placed in a dish containing a little water and left until the wound healed up. Usually not three drops of blood are lost, but sometimes when the operation seems to have been carried out in exactly a similar manner there is considerable bleeding. The point most needing attention is that the optic lobes be completely severed from the parts in front of them, before the latter are pushed forwards preliminary to removal; if the optic lobes be dragged in the least the animal does not recover normally, but exhibits either for some days or permanently a tendency to lean on one side. When, on the other hand, the operation has been neatly performed, the animal, from the moment that it is placed in the dish, sits up and breathes in a normal manner, exhibiting no tendency whatever to make spontaneous movements. In other cases movements may occur for some time after the operation, and if there has been much bleeding the frog squats down in an unnatural position for some hours. Sometimes too, from a cause which I have been unable to detect, the animal sits up at first in an abnormally erect position, and such rarely recover completely. My observations were made upon frogs which had assumed a normal and symmetrical position from the first, and were never commenced until at least three days had elapsed after the operation; in most cases not until after a week or more. Animals which did not exhibit lively reflex movements, and which were not capable of jumping vigorously, were not employed. The experiments were carried on upon autumn and winter frogs in the months of November, December, and January. The animals were kept in dishes containing a little water, changed daily, and in a room at from 13°—18° C.: they were not fed, as experience shewed me that for the week or two during which I desired to keep them, they did better without food; or at least without the exhausting struggle which the attempt to open their mouths called forth.

When an experiment was to be carried out, the animal was placed on the leaden platform in the manner already described and tracings taken of the throat movements at intervals of 15—20 minutes for from 1½ to 2½ hours. In this way the frog got used to its new circumstances, and a knowledge of the respiratory rhythm of the individual was obtained. The edges of the skin where they had united over the skull wound were then separated and a small crystal of pure sodium

chloride placed in contact with the cut end of the optic lobes. To do this it was necessary to clear away material which had accumulated in the cranial cavity, and this usually caused some bleeding. At first I contented myself with laying the crystal of salt on the ends of the optic lobes, and left it there to dissolve in the blood which collected in the cranial cavity. In my later experiments the salt was not applied until the blood had been sopped out by a bit of absorbent paper, and these have given more uniform results. If the "resistance" theory of the cause of the rhythm of the respiratory movements, so ably advocated by Rosenthal<sup>5</sup>, and I believe now generally accepted, be employed, the immediate and most marked effect on the respiratory centre may be thus expressed: the stimulation of the optic lobes by salt diminishes the resistance to the discharge of the inspiratory centre, usually leading for a time to a condition of tetanic inspiration, and increases the resistance to the discharge of the expiratory centre, leading to rare expirations which, when they occur, are of great violence.

Immediately after the salt is placed upon the optic lobes the animal exhibits violent movements, which render it impossible for some minutes to get a tracing. The majority of these movements, even if the animal be placed upon a dry table, are seen to be unequivocal swimming movements, varied occasionally with movements of progression, often of the "circus" type, probably from an asymmetrical position of the salt upon the optic lobes. The animal croaks frequently and these croaks are repeated at intervals for more than an hour. The throat is held for some time in a state of retraction (except during the croaks), *i.e.* there is a tetanic inspiratory discharge and complete inhibition of the expiratory centre. In four or five minutes very powerful expirations begin to occur, the throat during the long intervals being still held retracted. In ten or fifteen minutes the animal usually becomes quiet enough to allow a tracing to be taken, although for some half an hour or longer it continues to exhibit frequent spontaneous movements; but these have no longer the swimming character; it either jumps away or crawls off the edge of the lead plate. Henceforward there is a gradual change in a uniform direction: the active expirations become more numerous and less forcible: at last, somewhat suddenly, the throat passes from a state of retraction during the respiratory pauses to one of protrusion, and for a time the respirations are more powerful than normal, less frequent, and all of the "flank" type. This gradually passes

into the normal state. Three or four hours after the salt has been applied a "throat" respiration occurs now and then: these gradually become more frequent, and on the next day the animal is breathing in its usual manner, with on the average four or five "throat" respirations to one "flank," and all of about their usual force. In one or two cases the course of events has been different; after the animal had reached that condition in which the respirations are fairly numerous and all of the flank type, no further progress was made towards recovery: the respirations became feebler and rarer, at last barely perceptible, the skin on the back dried up, and death supervened at the end of about 24 hours.

The tracing represented in Fig. 8, Pl. VI., is a typical one, shewing the first effect of the salt. Seven tracings taken at intervals from 11 a.m. to 12.30 p.m. had shewn the animal to be breathing in a fairly normal manner, but the "flank" respirations less numerous and less marked than ordinary. At 12.35 a crystal of salt was carefully laid on the anterior cut end of the optic lobes. Croaks and swimming movements followed: then leaps and walking forwards. Respiratory movements were observed for a few seconds after the application of the salt, they then ceased entirely (except croaks) with the throat muscles in a state of retraction. At 12.43 respirations recommenced and, the animal being now sufficiently quiet, the tracing, Fig. 8, was obtained at 12.46. It shews the sudden and violent protrusions (active expirations) with which the throat retraction was now from time to time interrupted and the immediate return of the throat to its state of retraction (inspiration) after each of these expirations. Five of these respirations occurred in a minute: the average number of respirations in the seven preceding tracings was 64.5 per minute, varying from 72 to 57.5: the temperature had not altered a degree (having risen only from 16.1° C. to 16.8°). Until 1.10 this state of things remained practically the same, except that the throat protrusions became a little more numerous; but the animal was so restless as to make it impossible to get a continued trace. At 1.10 the number of respirations was 15 in a minute and they were less powerful, but the throat was still retracted in the intervals: this tracing is given in Fig. 9. At 1.22 the animal jumped out of the dish, and when replaced it was found that the periods of throat retraction had ceased: the respirations now occurred regularly at the rate of 31 per minute, were all of the "flank" type, but more powerful than normal: see tracing, Fig. 10. This condition lasted for some time: then one or two "throat" respirations per minute occurred,



and at 2.35 "throat" and "flank" respirations alternated nearly regularly, and the throat protrusions of the "flank" respirations had diminished to about their usual size (Fig. 11).

This example, selected from a number which essentially agree with it, shews clearly enough the influence of stimulation of the optic lobes upon the resistances to discharge of the inspiratory and expiratory centres, but a close examination shews that this is not the whole effect: the amount of discharge in a given time is also influenced, being diminished for the inspiratory and increased for the expiratory centre. Adopting the usual theory, this might be brought about either by an influence exerted upon the irritability of those centres, or by an alteration in the amount of stimulus acting upon them in a given time, or by both these together; of these three possible methods the first seems to me the most probable, as it is difficult to conceive how the stimulation of the optic lobes could affect the total amount of stimuli acting upon the respiratory centres. Adopting it provisionally, the above fact might be expressed by the statement that irritation of the optic lobes diminishes the irritability of the inspiratory centre and increases that of the expiratory. In Fig. 8 this effect is not very easily recognised, especially as regards the inspiratory centre, for it is almost impossible to form an opinion as to how much discharge in a unit of time is represented by the continued throat retraction periods. It is more obvious as regards the expiratory centre, for while the expiratory discharges are only between two and three times less numerous (5 to 12 or 15) in a minute than normal, they are certainly far more than three times as powerful. In the tracing given in Fig. 9 this is still more obvious, for while the expirations have now about their normal rate (15 per minute), they are considerably more powerful. In Fig. 10, when the throat retraction periods have ceased, we see clearly the less than normal amount of discharge from the inspiratory centre. The resistance to the discharges from this centre is still small, though now beginning to increase as the influence of the salt passes off, but nevertheless the discharges are less frequent than normal; and appear only to be called forth by that compression of the lungs by the expiratory muscles which I have already referred to as the probable cause of the greater throat retractions of the "flank respirations." If the amount of discharge were not less than with unirritated optic lobes, we should certainly, with the small resistance now present, have a "throat respiration" at the points marked *a* in tracing 10, where the horizontal line drawn by the lever

indicates that the throat was for some time at rest in its position of equilibrium before the next expiration (a "flank" one), indicated by the sudden descent of the curve, occurred. In Fig. 11 too, where the throat respirations have recommenced, their small number (in spite of the slight resistance to the discharges of the inspiratory centre, indicated by their feebleness) shews that the amount of discharge from the inspiratory centre in a given time is less than normal.

This general fact, of the diminution of the amount of discharge in the unit of time from the inspiratory, and its increase from the expiratory centre, is however much more readily recognised in some other cases, of which I will give one in detail (see next page), as it also shews the influence of feeble stimulation of the optic lobes, which illustrates the same fact. The time in this case, as in all the others, was taken for each tracing separately by an ordinary magneto-electric chronograph worked by a metronome; and the number of respirations per minute given in columns 4 and 5 was always counted for a full minute unless the frog moved away sooner, or some accident made part of the tracing illegible. In these latter cases the fraction of a minute during which the respirations were actually counted is indicated in column 4 by the numbers in brackets.

This experiment again shews quite clearly the influence of powerful stimulation of the optic lobes in diminishing the resistance to inspiratory, and increasing that to expiratory, discharges. The salt first produced tetanic throat retraction, with total inhibition of the expiratory centre. Then (Obs. 19 and 20) a state of things like that represented for another frog in Fig. 8 (the animal being in this case too restless to allow the corresponding tracings to be taken), viz. long periods of tetanic throat retraction interrupted by rare and violent expirations, after each of which the throat retraction was immediately resumed. This opposite effect upon the two centres is however most clearly shewn in Observations 21—31 (Figs. 15, 16, 17), where the feeble inspiratory discharges (except when increased in the manner before pointed out, by an immediately preceding expiration) contrast strongly with the powerful expirations, whose force shews that the impulses originating in the expiratory centre had to gather great head before they could overcome the resistance to their discharge. Inversely of course the feeble extent of the unaided inspiratory movements shews that the resistance opposed to inspiratory discharges was abnormally small.

## EXPERIMENT. Dec. 17, 1877.

Frog, a not full grown *Rana lentiginosa*, about size of *R. temporaria*. Central hemispheres and optic thalami removed on Dec. 11. Animal did well from the first. Kept in a room at temperature of from 12°—16° C. Put in experimental trough at 10.45 a.m., Dec. 17th.

Number of Observation.	Time.	Temp. C.	Total respirations per minute.	Flank respirations per minute.	Remarks.
1	a.m.	13.7°	45.5( $\frac{2}{3}$ )	12.5	Part of this tracing is given in Fig. 4.
2	11.15	13.9	45.0	8	
3	11.30	13.9	44.0	3	
4	12.0	14.1	43.0	None in the minute counted.	
5	p.m.	14.2	45.0	"	Cranial cavity opened by pushing apart the edges of the skin where they had united. Anterior part of cranial cavity sopped out with a cone of filter paper, and the anterior cut ends of the optic lobes were seen, covered by a translucent layer of "organising lymph." The latter was not removed, but an extremely small crystal of sodic chloride was laid upon it. The animal became restless for a few minutes; walked about; jumped once or twice; made no swimming movements; did not croak. No permanent throat retraction such as always follows powerful stimulation with salt.
6	12.30	14.8	45.0	"	
	12.33				
7	12.42	14.9	53.0	17.5	

Fig. 12. Here was obviously an increase to the expiratory discharge in a given time. The flank respirations are as powerful as when they occurred previously (Fig. 4), and considerably more numerous. How much of this was due to the salt is however problematical.

Number of Observation.	Time.	Temp. C.	Total respirations per minute.	Flank respirations per minute.	Remarks.
8	P.M. 12.50	14.9	51.0( $\frac{1}{3}$ )	16.0	Tracing essentially like that in Figure 12.
9	12.58	15.1	55.5	5.0	
10	1.5	15.1	53.0( $\frac{2}{3}$ )	8.5	
11	1.15	15.2	51.0( $\frac{2}{3}$ )	8.5	
12	1.25	15.2	50.5	3.75	
	1.30				<p>Fig. 13. Tracing now of normal type for this frog, which as preliminary tracings (Obs. 1—6) shew, was characterised by the small number of its active expirations.</p> <p>The layer of material on the cut ends of the optic lobes was removed carefully, and a small crystal of sodic chloride placed directly on their cut surface. Respiration ceased entirely for a short time. Animal performed circus movements; no swimming movements or croaking. Retraction of throat passed off in a minute and the respiratory movements recommenced; but each was now seen to be of the "flank" type, <i>i.e.</i> accompanied by a discharge from the expiratory centre. The brief throat retraction was no more than might be produced by simply handling the animal.</p> <p>Fig. 14. In the tracing it is not obvious that all the respirations were "flank," but the fact was established by direct observation of flanks and nares. Here there seems to be an obvious influence of the salt increasing the discharge from the expiratory centre in the unit of time; for the resistance (indicated by the extent of each movement) is about normal as seen by comparison with flank respirations of Fig. 4 taken from the same animal, while the number of expiratory discharges per minute is greatly increased. The state is comparable with that represented in Figs. 10 and 19, where the influence of a more powerful stimulation is beginning to pass off.</p>
13	1.35	15.2	55.5	55.5	

Number of Observation.	Time.	Temp. C.	Total respirations per minute.	Flank respirations per minute.	Remarks.
14	P.m.				As the effect of the salt was now obviously passing off a somewhat larger crystal was placed in contact with the cut ends of the optic lobes. The animal croaked; clonic spasms set in and lasted for a few seconds; then swimming movements. Throat powerfully retracted and no respiratory movements seen until 2.33, when one occurred; the next at 2.35, followed by several croaks. Animal remained restless for some time, so that no tracing could be obtained.
15	1.40	15.2°	50.0	48	
16	1.45	15.2	50.0	36	
17	1.50	15.2	48.0	37	
18	2.0	15.6	50.0	30	
	2.20	15.7	50.0	27	
19	2.38	15.7	About 16	About 16	An attempt made to obtain a tracing was frustrated by the restlessness of the animal. The one or two curves that were obtained shewed that it was breathing as the frog represented in Fig. 8, that is with long throat retraction pauses, and powerful expiratory discharges at intervals. Animal too restless to allow a satisfactory trace to be taken. Character of respiratory movements same as in Obs. 19.
20	2.45	15.8	About 8	About 8	
21	3.0	16.0	62	6	

Fig. 15. Here we find some resistance to the inspiratory centre beginning to arise. The throat retraction is no longer tetanic, but while still, on the whole, retracted. The throat has very feeble rhythmic movements of return towards its position of equilibrium, but the resistance permitting these rhythmic relaxations is so feeble that it is overcome almost at once, long before the throat has returned to that position; thus very feeble throat respirations are produced. With reference to the expiratory discharge, the rise of the curve at *a* is not due to a respiratory

Number of Observation.	Time.	Temp. C.	Total respirations per minute.	Flank respirations per minute.	Remarks.
	p.m.				
22	3.5	16.0	45.0 (?)	3	movement proper, but to the fixing of all the muscles of its body by the animal before making the violent expiratory effort. By this fixation it sits up rigidly and more erect, and the lever, following the throat as it rises with the rest of the head, produces the curve ascent at <i>a</i> . Similarly the descent at <i>b</i> is not due to a throat movement proper, but to a sudden relaxation of its muscles and collapse of the whole animal after its violent respiratory effort. This fixation of all the muscles of the body before the violent expiratory discharge is very common in frogs in the most powerful stage of optic lobe irritation.
					The "throat" respiratory curves too feeble to be counted with certainty.
					If there be any error, the number is too large.
					Ditto.
23	3.15	16.1	49.0 (?)	4	Ditto.
24	3 (?)	16.1	49.0 (?)	4	Traces essentially like Fig. 15.
25	3.35	16.0	48.0	3	Fig. 16. "Throat respirations" now well marked, though still very feeble.
26	3.45	16.0	52.0	3	Rise of curve at <i>a</i> produced in same way as similar rise in Fig. 15. The irregularity in the ascent after the expiration is of course due to imperfect action of the writing point.
27	4.5	16.1	59.0	3	Fig. 17. Compare with Fig. 9, from which it differs in the fact that it presents small rhythmic movements during the throat retraction periods, the inspiratory discharge not being here quite tetanic. The breaks seen in the ascending limb of the larger curves in each figure depend probably on the fact that the first part of the ascent represents the passive return of the hyoid to its position of rest; the part above the break is due to contraction of hyoid elevating (inspiratory) muscles.
28	4.30	16.1	47.0	12	A return to the type of respiration seen in Obs. 21-27. (Figs. 15 and 16.)
29	5.0	16.2	47.0	2	



Number of Observations.	Time.	Temp. C.	Total respirations per minute.	Flank respirations per minute.	Remarks.
30	p.m.			2	Animal pushed away the lever and crawled out of the dish soon after the tracing of Obs. 31 was taken. When it was replaced and things were made ready for the next tracing it was found that the stage characterised by the long respiratory pauses with throat retraction had passed off. Fig. 18. Respiration now fairly normal, except that the "flank" respirations were more numerous.
31	5.30	16.2 16.4	49.0 43.0	4	
32	6.0	16.8	37	15	
33	6.10	16.8	36	32.5	
34	6.30	17.1	37	34	Fig. 19. Almost exactly like Fig. 10. Abnormally powerful and frequent "flank respirations," with rare (3.5 per 1') throat respirations. This stage almost invariably precedes such a stage as that represented in Fig. 18, and gradually passes into it, the "flank respirations" becoming less powerful and less frequent, and the "throat respirations" more frequent until the normal respiratory modus is resumed. The skipping of this stage for a time in this case (as shewn in Fig. 18) is therefore probably to be ascribed to the sensory stimuli applied to the animal, in replacing it in the dish, readjusting the lever, &c. Animal having now reached the stage which I knew from previous experiments would pass with extreme slowness into the normal condition, the observation was discontinued until the next day.
35	Dec. 18 12.30 p.m.	16.4	52	18	Fig. 20. Compare with Fig. 4 from same frog before the salt was applied. On Dec. 19 several tracings were again taken from the frog; these were quite normal, but the active expirations more numerous than they were on Dec. 17 before the salt was applied. The difference was however not greater than may be seen on different days in frogs on which no experiment has been performed.

Turning now from the resistance opposing an individual discharge, to the total amount of inspiratory or expiratory discharge taking place in a given time, we find in the tracings, I think, evidence of the proposition stated above, that the total inspiratory discharge, in say a minute, is diminished and the expiratory increased. Taking the expiratory centre first, we find that in this frog before the salt was applied it was hardly active. In Obs. 4, 5, and 6 it did not discharge once a minute, yet the resistance to it was not great, as shewn by the only normally large discharge when it did occur. When the optic lobes were very feebly stimulated active expirations became much more numerous (Obs. 7—12), the resistance still remaining practically unaltered: but it may be doubted whether this increase was not due rather to the general irritation of the frog in holding it to open the cranial cavity, &c., than to the immediate influence of the small salt crystal, acting through the layer of material covering the ends of the optic lobes. When however this layer was removed, and a very small salt crystal carefully applied directly to the optic lobes, a much more definite result was obtained: there were 55·5 expiratory discharges in a minute (Obs. 13), each of about its usual amount, so that the resistance did not seem to have been affected by this amount of stimulation; the slightness of which was further evidenced by its not calling forth croaks or swimming movements. The state of things is in fact very like that seen later when the influence of more powerful stimulation was beginning to pass off. When more salt is applied so as to affect also the resistance to the discharge of the centre it is much more difficult to make an estimate of the amount of discharge in a given time, since it is hard to get even an approximate idea to how many of the smaller discharges one of the larger is equivalent. When the effect of the salt begins to pass off however, and the resistance to the expiratory discharges becomes again somewhat less, it becomes also easy to see that the total expiratory discharge in a given time is above the normal. In Observations 33 and 34, *e.g.*, there are more than thirty expiratory discharges in a minute, and each of more than normal amount.

With reference to the inspiratory centre, the influence of the stimulation of the optic lobes upon its total discharge in the unit of time is also difficult to estimate in the stage of greatest stimulation; the throat is then tetanically contracted, and one cannot say to how many normal discharges a given period of this retraction is equivalent. But when this condition begins to pass off and feeble throat movements appear, it seems tolerably plain that they represent less than the normal

amount of discharge from the inspiratory centre. In Obs. 21 (Fig. 15), where the total number of respirations is 62 per minute, it might be supposed perhaps that in spite of the feebler resistance overcome, as indicated by their small extent, the increased number (as compared with the average number of inspirations, 45 per minute, before the salt was applied) made up for the deficiency in size. But in Observations 22—28 it is seen that the average number of inspirations per minute is under fifty, and here the increase in number of 3 or 4 per minute certainly does not compensate for the diminution in amount of each. Were the resistance as small as these feeble movements shew it to be, and at the same time the total discharge from the inspiratory centre anything approaching its normal amount (see Fig. 4), the number of inspiratory discharges per minute would certainly rise far above 50.

So far then as stimulation of the optic lobes is concerned, we have, I think, sufficient evidence to justify the propositions which I stated, before giving the experimental details upon which they were based. It would have been, no doubt, desirable to observe if, when the full effects of optic lobe stimulation had manifested themselves, the severance of those parts from the medulla removed those effects. But as Kramszük<sup>8</sup> has pointed out, a frog whose brain in front of the medulla is removed, will not stay still a moment; it crawls about for hours, presenting a striking contrast to the motionless frog with its optic lobes. It is therefore impossible to get a respiratory tracing from such frogs, at any rate in their normal position, until some hours after the operation has been performed, when it would be useless for comparison; and then, moreover, as I pointed out above, the animals seem thoroughly exhausted and in an unphysiological state.

In the facts, however, that the characteristic results of the stimulation were not called forth, at least to nearly their full extent, unless the salt was so applied as to excite centres (those for croaking and swimming) known to be situated in the optic lobes, and that when these centres were excited the characteristic effects on the respiratory movements never failed to appear, we have, I think, strong presumptive evidence that these effects depend on stimulation of an optic lobe centre. That they do not depend upon the salt soaking through and attacking the respiratory centres in the medulla directly, seems to be shewn by the rapidity with which the application of the salt to the optic lobes is followed by the respiratory disturbance; within a few seconds tetanic throat retraction is usually seen. That they do not depend merely on the stimulation of the cut ends of fibres which

passed from the fore brain through the optic lobes to the medulla oblongata, but on the stimulation of an optic lobe centre, seems to be shewn by the fact that when the amount of salt applied is so small as not to excite other optic lobe centres (Obs. 13 in the experiment given in detail), the characteristic respiratory effects are also in great part wanting. The salt appears to be required in sufficient quantity to penetrate into the optic lobes, mere stimulation of the cut surface not being sufficient, as it would be if the effect were due to irritation of fibres passing as above supposed.

Another objection may be raised to the view which I have taken, an objection based on the long continuance of the effect of the stimulation; which may be supposed to indicate rather the paralysis, due to injury, than the excitement of a respiration-regulating centre in this part of the brain. The reasons which lead me to ascribe the effects to a stimulation rather than a paralysis are these. First, the complete recovery of the animal in most cases; this shews that there has been at least no actual destruction of the regulating centre, such as might be supposed to be brought about by the action of the salt. Second, the action on the other centres of the optic lobes is obviously excitant; the croaking, swimming, and locomotor centres are all stirred up to activity, and it is *a priori* improbable that the same influence which excites them will paralyse the respiration-regulating centre. Third, the activity aroused in some other optic lobe centres by the salt lasts nearly as long as the effects on the respirations. The swimming movements soon cease; but the animal has a tendency to crawl about for nearly an hour in many cases, not continuously it is true, but at short intervals; so that we have a tolerably long-lasting irritation of the centre exciting and coordinating the locomotor apparatus. The croaks, too, occur at intervals in many cases for more than an hour and a half; but the centre whose excitement lasts longest is that inhibiting the reflex actions of the cord (Setschenow<sup>9</sup>). Again and again I find in my notes that the time when the animal begins once more to shew reflex irritability is just when the influence of the salt upon the respiratory movements is beginning to pass off, or after it has commenced already to weaken. I find it nearly always noted that the animal, after having borne the lever without trouble or readjustment for a considerable time (commencing from the cessation of the crawling movements), "now begins again to shew signs of reflex irritability," pushing the lever away, or sometimes crawling off the edge of the dish, such reflexes having been suppressed hitherto from the time of application

of the salt. In the experiment given in detail, *e.g.*, the frog gave no trouble from 2.45 p.m., when (in this particular case) the crawling movements had ceased, until after 5.30 p.m., when it pushed away the lever and crawled out of the dish, shewing that the excitement of the inhibitory centres of Setschenow had lasted until that time; and immediately after this, as the next tracings shew, the frog began its steady progress back to a normal mode of breathing. The long continuance of the respiration-modifying effects of the salt applied to the optic lobes is then not by any means inconsistent with the view, more probable on other grounds, that its action is stimulant rather than paralyzing. On the other hand, the almost invariable connection which I have found between the time when the inhibitory effect of optic lobe stimulation on the general reflex actions of the animal begins to pass off and the time when the respiratory effects begin also to pass off, suggests strongly a close relationship between this respiration-regulating centre and the general reflex-regulating centre, even should the two not be identical. Two experiments with injection of quinine into the dorsal lymph sac, made with the view of seeing whether the quinine excited the respiration-regulating centre, as it has been stated to excite the reflex-inhibiting, gave me no definite results; but the animals were not in a satisfactory state, my winter supply of frogs having run short.

Several theoretical points of interest are suggested by the results of the foregoing experiments, but most require further investigation, and I will only refer to one here. The rhythmic alternation of the innervation of expiratory and inspiratory muscles may be accounted for, either by supposing the existence of independent, though closely related, inspiratory and expiratory nervous centres (Budge), or by the hypothesis of a single respiratory centre capable of discharging to either group of muscles according to circumstances. Rosenthal<sup>6</sup> discusses the question and decides in favour of a single centre, with two resistances in relation with it; one intercalated on the road to the inspiratory nerves, and the other on that to the expiratory. Whether an inspiratory or an expiratory discharge shall issue from the centre will then depend on the ratios of the two resistances; and since in most mammals the expiratory resistance is greater, no active expirations occur at all in their normal breathing. The results of optic lobe stimulation shew that this hypothesis will not hold good for the frog, since they make it obvious that impulses tending to produce expiratory discharges have not the path to the inspiratory muscles open to them.

For, during stimulation of the optic lobes, the resistance on the latter route is abnormally small, as shewn at first by the tetanic contraction of the inspiratory muscles, and later by the feeble throat respirations, while the resistance on the expiratory route is abnormally large. If the impulses originated in the same centre and took merely the more open road, there could arise no such accumulation as that which finally overcomes the enormous resistance to the expiratory discharges and calls forth the powerful expiratory movements depicted in Figures 8, 15, 16, &c. We must assume that the impulses which finally break forth along the expiratory nerves are not able to travel into the inspiratory nerves; and that we have really two distinct centres, one for inspiration and one (normally less easily discharging) for expiration, and that each has its own stimulus and generates its own nervous impulse which can travel only to its own set of muscles, quite independently of the resistance opposed to discharge from the other centre.

This seems also a necessary deduction from the normal mode of breathing in the frog, for were both inspiratory and expiratory muscles innervated from the same centre no "flank respirations" could occur, the resistance along the paths to the muscles producing the throat respirations being less than that in the paths to the expiratory muscles of the flanks: the nervous impulses would, so to speak, be constantly "tapped" and never accumulate so as to discharge to the flank muscles. As these latter, however, do contract at intervals, this tapping clearly does not take place with respect to the impulses generated for the expiratory muscles, which finally gain head (the throat movements not renovating the air in the lungs as the inspiratory discharges with passive expirations do in the lungs of the mammal) until they overcome the opposing resistance. In what manner the immediately succeeding greater inspiratory discharge is brought about, I have already tried to explain.

In conclusion, I would point out that the results of chemical stimulation of the optic lobes in the frog seem to agree very well with the results of electrical stimulation of the corpora quadrigemina in the mammal, as described by Ferrier'. His account, it is true, is somewhat indefinite, and is given as a confirmation of results of Danilewsky's, which the latter did not obtain. Ferrier's words, speaking of the effect of stimulation of the interior of the corpora quadrigemina, are "the respiratory rhythm is also altered in a marked degree; irritation causing a deep inspiration, followed by prolonged and powerful expiratory efforts." Here it seems probable that between the expira-



tions the chest was in a tetanic inspiratory position; and if so, the state of things will almost exactly correspond with the results of chemical stimulation of corresponding parts in the frog.

NOTE. The very small secondary curves seen in some of the tracings in Figs. 4, 10, 20, &c., are due to vibrations of the stand carrying the lever, the floor of my work-room being unfortunately not very steady.

BALTIMORE, March 18, 1878.

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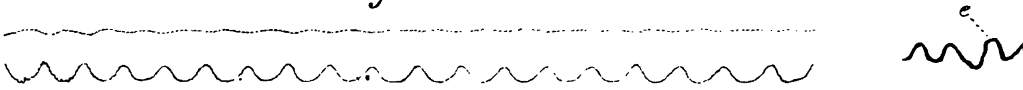
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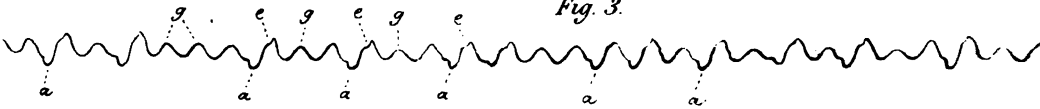




*Fig. 1.*



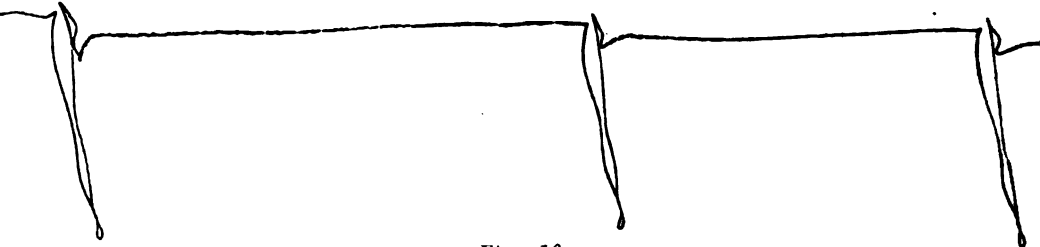
*Fig. 3.*



*Fig. 4.*



*Fig. 8.*



*Fig. 10.*



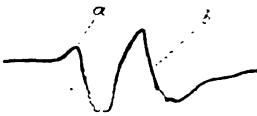
*Fig. 11.*



*Fig. 12.*



*Fig. 13.*



*Fig. 14.*



Fig. 2.

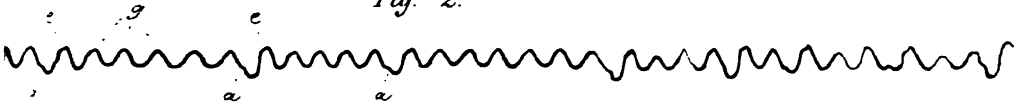


Fig. 5.

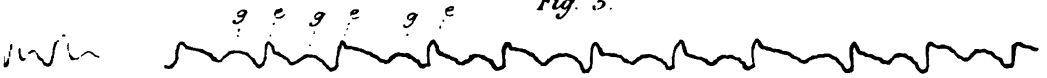


Fig. 6.

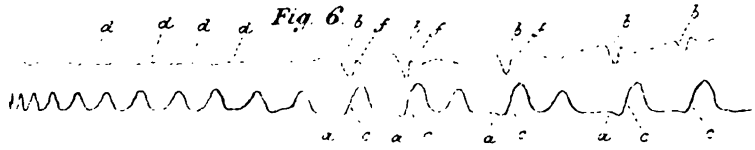


Fig. 7.

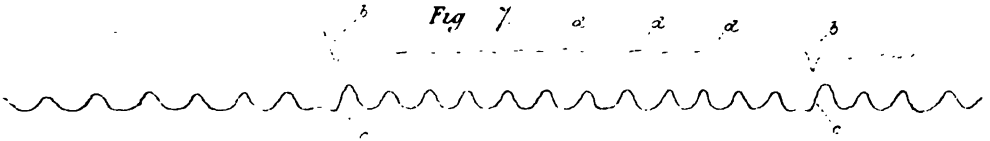


Fig. 9.



Fig. 16.

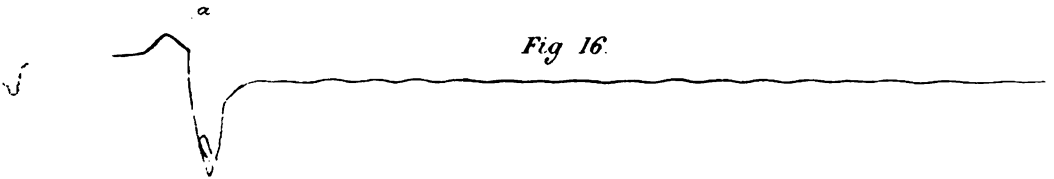


Fig. 17.



Fig. 15.



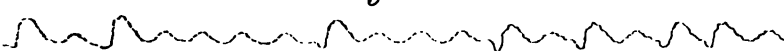
Fig. 18.



Fig. 19.



Fig. 20.







[*From the Journal of Physiology*, Vol. I. Nos. 4 and 5.]



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**THE DEVELOPMENT AND REGENERATION OF THE  
GASTRIC GLANDULAR EPITHELIUM DURING  
FOETAL LIFE AND AFTER BIRTH. By HENRY  
SEWALL, B.Sc., *Fellow of the Johns Hopkins University, Baltimore,  
U. S. A.* (Pl. XII.)**

A PROLONGED investigation made on the stomachs of adult animals having failed in its object of giving me some insight as to the functions of the different cells in the glands of the stomach, I determined to take up the question in another way; and by carefully examining the stomachs of embryos to seek if there was any correlation between the differentiation of certain cells in the embryo stomach and the first appearance of functional activity. This method, the results of which are here detailed, has not done all I had expected from it, but has, nevertheless, I venture to hope, not been entirely fruitless.

The animal mainly used in the embryological part of the work was the sheep, lamb embryos being those most easily obtainable in sufficient number. From ewes slaughtered at the butcher's I easily got large numbers of embryos in various stages of development, and ranging in length from less than an inch up to the length (19-20 inches) of the mature foetus. Between these limits individuals corresponding to intervals of less than an inch in length were obtained. The length was measured from the anterior end of the forehead to the posterior part of the trunk; not having been able to accurately find out the age of the embryos, I can only roughly indicate it in the following pages by giving the measurements as taken in this way.

In the fourth or glandular stomach of the adult sheep, the mucous membrane projects in numerous flaps, which though not so marked as those of the psalterium, yet resemble them in being permanent, and so differ from the temporary 'rugæ' of the human and many other stomachs, to which in other respects they appear to correspond. Histologically the majority of the glands in this mucous membrane agree with those of other mammals in possessing 'central' cells (*Hauptzellen*) and 'ovoid' cells (*Belegzellen*), but the latter seem aggregated in greater numbers in the deep parts of the gland than in most

stomachs which I have examined. In addition to the above there is the usual pyloric zone, containing glands with a wider lumen, and lined with cells of one kind only.

The embryos were obtained not later than four hours after the death of the mother, and the fourth stomach carefully dissected out, except in the case of very small embryos. In the stomach there was always a quantity of fluid containing much mucus, which seemed to become more abundant as development went on, so that in the oldest embryos the liquid containing it was extremely thick and glairy. When wanted for microscopic examination the stomach was placed for 24 hours in 0.1 per cent. solution of chromic acid: from this it was put into 0.3 per cent. solution for 24 hours; then transferred to 70 per cent. alcohol for 24 hours, and finally put in 95 per cent. alcohol. The smaller embryos were hardened whole, the peritoneal cavity being previously opened. After this hardening, sections were made from the pyloric and other regions of the stomach, and stained in a manner essentially agreeing with that described by Heidenhain<sup>1</sup>. They were placed for about 20 hours in an extremely dilute watery solution of aniline blue; then placed in dilute glycerine for about a week in an open watch-glass, so as to allow the absorption of acetic acid vapours given off from a neighbouring watch-glass placed under the same bell-jar. The best results were obtained when the aniline solution was of such strength that its colouring matter was completely taken up by the sections.

As to the general course of the earlier stages of the development of the gastric glands, I can in the main confirm the statements of Brand<sup>2</sup>, so far as I can gather them from the abstract published in the *Centralblatt*: as also in a general way those previously made by Laskowsky<sup>3</sup>, who is however very meagre in details.

Primarily the epithelial lining of the embryonic alimentary canal is here, as elsewhere, a single layer of large columnar cells, seated upon and sharply marked off from a branched layer of the mesoblastic cells around them. The nuclei of the lining hypoblast cells are very large, granular, staining deeply, and round or oval. This condition does not however last long.

Sections taken from different parts of the alimentary canal of a lamb embryo, five-sixths of an inch in length, shew local differences in the lining epithelium. In the intestine it still consists of a single layer of large cells (Pl. XII. Fig. 1), while in the stomach and œsophagus the cells are so closely crowded that many are pushed away

to a greater or less extent from the basement membrane, thus giving the epithelium the appearance of being composed of several layers.

I must therefore take exception to Brand's statement, that the epithelium consists originally of several layers of cells: this is an appearance which only comes some time after the closure of the digestive tube, and sooner in the stomach and œsophagus than in the intestine. Laskowsky describes but a single original layer of cells. The change is probably due to cell-multiplication in the hypoblast, and is accompanied with alterations in the individual cells. Where the epithelium is still arranged in a single layer the nucleus is situated in the centre of the cell, and the latter stains uniformly throughout (Fig. 1). Where, on the other hand, the cells lie in more than one layer, where in fact active cell-multiplication is taking place, the nucleus lies nearer that end of each cell which is turned away from the mesoblast, and this end of the cell or the nucleus itself is all that picks up the aniline blue (Fig. 2, *a*; also Figs. 3 and 4).

This cell-multiplication takes place at first uniformly over the interior of the fourth stomach, but soon unequal growth begins and the epithelium projects along various lines into the cavity of the stomach, thus forming ridges. Sections of these shew a single layer of hypoblast cells borne upon processes of the mesoblast cells beneath: as they grow, the epithelial cells and the supporting branched mesoblast cells multiply *pari passu*. The first protrusions of the mucous membrane are few in number, and as seen in cross section, contain a relatively broad core of mesoblast; they become the large permanent flaps of the mucous membrane of the adult stomach, and probably the rugæ of other stomachs. They are seen in the section depicted in Fig. 3.

The primary process in the development of the glands is essentially a repetition of that occurring in the development of the above-mentioned flaps. Synchronous multiplication of hypoblast cells and of supporting mesoblastic corpuscles along definite intersecting lines is the first evidence of glandular formation (Fig. 3, *a*). Projections, which may be called 'gland processes' are thus formed; as seen in section they present a slender central mesoblastic core, from which branches penetrate between the epithelial cells covering it; Fig. 4 represents one of these processes more magnified. As Brand states, these outgrowths commence earliest and develop fastest in the pyloric region of the stomach, but he says that the first outgrowths are in the form of villi or papillæ, which are afterwards united by outgrowths from their

sides and by upgrowths from the membrane between their bases, so as to close in the gland pits. In this latter point I cannot agree with him: my sections shew distinctly that from the first the outgrowths are not papilliform, but take place along continuous lines of greater or less extent, giving rise to ridges, which intersect in all directions, and which are cut sometimes vertically, sometimes longitudinally, and sometimes obliquely in the sections. Immediately beneath the epithelium is a well-marked layer of mesoblast cells, flattened parallel to the surface and forming a very distinct basement membrane (Fig. 4, *a*).

Keeping pace with the superficial growth of the stomach-wall, new protrusions of the mucous membrane appear budding out either from the sides of those already formed or from the hollows between them (Fig. 5). This multiplication of outgrowths goes on, making the spaces between the intersecting ridges smaller and smaller, until they are finally reduced to minute canals, which become the lumens of the glands. It may be readily understood from Fig. 5, *a*, how several glands may come to open into a single vestibule by the formation of new outgrowths between two which have already attained a considerable size, the new outgrowths not growing out as far as the original ones, and so leaving a small pit which becomes the common vestibule.

In a sheep's embryo about 16 inches long the glandular formation seems to be completed. Blood-vessels appear early in the mesoblastic cores of the gland processes, as extensions from vascular areas lower down (Fig. 5, *g*). I have first found vessels containing red corpuscles in the gland processes of an embryo  $6\frac{1}{2}$  inches in length.

I have also had the opportunity of examining the stomachs of foetal pigs, kittens and puppies, though not in as large numbers as those of the lamb. In all, the appearances indicated a mode of gland formation exactly similar to that described above. In the cat and pig the first extensions of the mucous membrane become the rugæ, and the gland processes proper appear later. At birth, however, the glands in these animals are very immature, being much shorter and relatively thicker than those of an adult, while in the lamb they attain their full development some time before birth.

The recognition of the first differentiation of the primitive hypoblast cells into the proper secreting gland cells is not always easy, since individual cells are apt to present considerable variation from the type-form as to shape, staining, granulation, &c. In early stages the cells on the gland processes and between them, which we may

call the "embryonic gland cells," are tolerably uniform in character, and are distinct from either 'ovoid' or 'central' cells, and from the adult pyloric gland cells. Laskowsky<sup>3</sup> simply says that in the process of development the cells at the bottom of the gland-pits become differentiated into the true secreting elements. I have not been able to discover the statement, attributed to him by Nussbaum<sup>4</sup>, that there are no 'ovoid' cells in the embryonic stomach. At any rate in the sheep embryo they appear comparatively early, and increase in number up to the time of birth, when they are relatively quite as numerous as in the adult stomach. In an embryo about  $5\frac{1}{2}$  inches long one finds one or more cells at the bottom of a gland to have taken on new characters; they are larger, more oval, stain better, and are more granular than the 'embryonic' gland cells; and are to be regarded as the first formed 'ovoid' cells, although they differ somewhat from the fully formed 'ovoid' cell of the adult. These cells are sometimes enclosed in a close network of processes from mesodermic cells, in other cases not. Fig. 5, *b*, shews 'ovoid' cells, (a little too sharply defined,) from an embryo eight inches long. The shape of these cells is subject to much more variation than that of the corresponding cell in the adult, and in various specimens gradations in every character may be found between them and the undifferentiated embryonic gland cells, such as is represented at *c* in the same figure. The 'ovoid' cells first appear in the deep parts of the glands; and in older embryos they may be traced on, assuming more and more their completely developed characters, and becoming more numerous and extending farther up the gland. The numerous transitional forms between the 'ovoid' and 'embryonic' gland cells seem to indicate that the former arise, at least in part, from a differentiation of the latter. I will afterwards mention reasons which lead me to think that they may have also another origin. When once formed, I have seen appearances which lead me to believe that the 'ovoid' cells also multiply by fission.

A wholly unexpected phenomenon was the appearance of ovoid cells in the pyloric region of the embryo stomach. At first this was thought to be simply due to a mistake in the locality of the stomach from which the piece was taken for the preparation of the sections; but great care was afterwards exercised upon this point, and undoubted ovoid cells were obtained from the pyloric region of stomachs of sheep embryos of all lengths from  $4\frac{1}{2}$  to  $11\frac{1}{2}$  inches. Fig. 6 shews ovoid cells (*b*) from the pyloric region of the stomach of an embryo 8 inches in length. They

appear earlier in the pyloric region than elsewhere, in accordance with the general developmental precocity of that part. In embryos 12 inches and upwards in length the ovoid cells have disappeared from the pyloric glands, which are then lined by their characteristic epithelium; what becomes of the ovoid cells, unless they divide up into the pyloric gland cells, I do not know.

The central cells (*Hauptzellen*) are first differentiated so as to be definitely recognisable as such in sheep embryos about  $5\frac{1}{2}$  inches long, but they first appear abundantly in specimens from 7 to 8 inches in length. They still differ from the adult central cells in their greater size and larger nuclei; from the 'embryonic' gland cells they differ in their much less columnar form and in the absence of the darkly staining material always aggregated around the nucleus of the former. They are formed, I believe, by a differentiation of the embryonic cells, accompanied by division. At *d* in Fig. 5, a series of developing central cells is depicted. In the embryonic sheep comparatively thick connective tissue processes extend between the gland cells, giving rise to a somewhat denser framework than that which has been described by Watney<sup>5</sup> in adult stomachs.

In the foetal cat and pig differentiation is much more tardy. In a cat embryo  $3\frac{1}{4}$  inches in length no central cells could be recognised: in one  $5\frac{1}{4}$  inches long the glands contained for the most part ovoid and embryonic cells alone, few that could be called with certainty central cells having as yet appeared. In the cardiac part of the stomach of a pig 7 inches long ovoid cells abounded, but no well marked central cells could be recognised: there were no ovoid cells in the pyloric glands, which were fully developed.

In connection with the histological work experiments were carried on to ascertain the proteolytic power of the embryonic stomach. For this purpose the stomachs of sheep and other embryos were removed within a short time of death, washed with 0.75 per cent. NaCl solution, and placed in from 50 to 100 cc. of 0.2 per cent. HCl for 15 or 20 hours. The extract was then filtered, and an equal bulk of 0.2 per cent. HCl added to the filtrate. Cubes of coagulated egg albumin were then added, and the whole placed in a warm chamber kept at 38° C. The results are given in a condensed form in the accompanying Table I, see p. 327. It will be seen that the stomach of the kitten nearly at term, shews no digestive action upon proteids; the same is true of the stomachs of pig embryos from 5—7 inches in length. On the other



Table I.

No.	Embryo.	Length in inches.	Treatment of stomach mucous membrane.	Result.
1	Sheep.	17½	24 hours in 0·2 per cent. HCl: filtered: to filtrate added equal bulk of 0·2 per cent. HCl and cubes of boiled white of egg, and put in hot chamber. Some of filtrate neutralised and added to milk: placed in warm chamber.	Albumin partly dissolved in 10 hours. Wholly in 48.
2	"	17	As above.	Milk coagulated in three hours.
3	"	15½	As above.	Albumin nearly all dissolved in 48 hours. Milk coagulated in between 3 and 4 hours.
4	"	15½	As above, except none neutralised to try with the milk.	Albumin in great part dissolved in 48 hours. Milk coagulated in 3—4 hours.
5	"	14½	As above.	Albumin swollen, but dissolved only at the edges even after 76 hours.
6	"	11	Two stomachs treated as above.	Albumin nearly all dissolved in 76 hours.
7	"	9	"	"
8	"	7½	As above, but with 75 cc. of dilute HCl in all, instead of 150 cc.	Albumin about half dissolved.
9	Fig.	5—7	Several embryos. Treated as 1 above.	Albumin scarcely affected.
10	Cat.	3½	As above, except milk test.	No perceptible action on either albumin or milk.
11	"	5½	Three stomachs treated as above, but boiled fibrin used instead of albumin.	Albumin not affected. No digestion.

Comparative experiments made with 0·2 per cent. HCl above showed the albumin in no case affected.

The fluid contained in stomachs 6, 7 and 8 was mixed and acidulated with 0·2 per cent. HCl and placed in warm chamber with cubes of coagulated albumin. These were partly dissolved in 76 hours.

hand, the extracts of stomachs taken from sheep embryos even some time before term, and when only 6—7 inches in length, shew a considerable proteolytic power; as also the ability to curdle milk in a neutral solution: this last reaction however was traced back but a short way. The statements of Hammersten\*, as to the want of digestive ability in the stomachs of foetal and newly-born animals, cannot therefore be made universal. The different results are satisfactorily explained when we consider the marked precocity in the differentiation of the cells in the stomachs of sheep embryos as compared with those of the cat, pig, and dog.

The fluid which has already been mentioned as present in the fourth stomach of sheep embryos shewed a very perceptible digestive power when acidulated. This fluid is clear, gives an abundant precipitate of mucus with acetic acid, but no peptone reaction. It is uniformly neutral to test-paper, and thus gives evidence of the secretion of a pepsin-containing fluid, without that simultaneous secretion of free acid which characterises normal gastric juice. It points thus to a conclusion, which many other vital phenomena of the stomach support, that the formation of pepsin and of free acid in the stomach are the results of different chains of events, and have no necessary connection.

To fix with certainty the functions of the various cells, ovoid, central and pyloric, in the stomach glands demands varied and extensive research. The results obtained by my work, so far as they go, all point however to one conclusion. In the stomach of the embryo sheep ovoid cells appear very early, but no digestive power could be found to exist until a considerably later period, when central cells were also well differentiated. The glands of the foetal pig 7 inches long, and of the foetal cat 5½ inches long, contained many ovoid cells, but few or none which could be called with certainty central cells. In both the pyloric glands were well formed, and their cells specialised.

There can, then, I think, be little doubt, that whatever part the ovoid cells may play, they are not the immediate pepsin formers; and similar reasons seem to exclude also the cells lining the pyloric glands. The central cells in the sheep embryo are recognisable some short time before I could detect any proteolytic power in the stomach extract, but on their first appearance they are very few in number, and for some time increase but slowly. In embryos about 7 inches in length they increase rapidly in number, and it is just at this period that definite evidence of digestive power can be obtained. I believe then that by the combination of histological and physiological observations, as de-

tailed above, we get very strong evidence in support of the views of those who, on other grounds, have maintained that the central cells are the immediate pepsin formers (Haidenhain<sup>1</sup>, Grützner<sup>7</sup>, Święcicki<sup>8</sup>, Partsch<sup>9</sup>, Ebstein<sup>10</sup>), and against the view that the ovoid cells are the essential pepsin producers maintained by Herrendörfer<sup>11</sup> and Nussbaum<sup>12</sup>. On the other hand, the evidence brought forward by me tends to shew that Herrendörfer<sup>11</sup> and Nussbaum<sup>12</sup> are correct in denying the pepsin forming power of the pyloric glands, which has been maintained by Grützner<sup>13</sup> and Klemensiewicz<sup>14</sup>.

I have already mentioned that, although undoubted transitional forms exist between the embryonic gland cells and the primary ovoid cells, there was reason to think that the latter also originated in another way, viz., from the immigration of cells from the mesoblast. In the branching and growing cells of the embryonic mucous membrane it is not always easy to fix the boundary line of hypo- and mesoblast. Hitherto only cells lying distinctly within the latter layer have been spoken of as ovoid cells; but even these are often surrounded by thick processes from the mesoblast. In addition to these, however, one often finds in the mesoblastic layer below the glands, cells which, were their position in the other layer, would be unhesitatingly called ovoid cells. I have found them best marked in the embryo cat. In such about 5½ inches long, and just before birth, the glands are imbedded in a thick layer of connective tissue, which contains many cells, varying from those having the appearance of undoubted connective-tissue corpuscles to those having the features of ovoid cells. The appearances presented by a lamb after birth led me to investigate the origin and fate of the ovoid cells further. The animal had been born eight hours, and during that time had received no nourishment. Sections from its stomach shewed the glands to be remarkably poor in ovoid cells, many glands containing only central cells, but these of normal hungering character. In seeking an explanation of these appearances, so different from those of the stomach just before birth with its abundant ovoid cells, and from those seen in normally fed animals, I thought it possible that there might here be a stage of gland life made apparent by slow and languid vital processes going on at the expense of a continually diminishing amount of store material and energy during the eight hours of the lamb's life. I therefore made an attempt to imitate the conditions, and with some striking results. Adult animals (cats and dogs) were taken, and suffered to fast for some days: the stomach was then mechanically excited by pieces of india-rubber tubing introduced through the gullet and

left in it: after six hours the animals were killed, and parts of their stomachs were hardened for examination, and the sections stained with aniline blue, as before described.

The sections from non-pyloric parts of the stomachs of animals so treated shewed glands of various appearances, which may however be arranged in three groups. A few were quite free from ovoid cells, and were larger and thicker than the rest, the central cells filling them being great and clear like typical hungering cells. They resembled very closely the cells from the stomach of the lamb above mentioned. More numerous were glands also devoid of ovoid cells, but thinner than the above; their cells being smaller, more shrunken, and staining more deeply than ordinary central cells (Fig. 7).

The greater part of the mucous membrane, however, was filled with glands which were slender and shrunken, particularly towards the neck, and which stained deeply. They contained a variable number of ovoid cells, but frequently no central cells, especially towards their outer ends. The central cells when present were quite normal, except that they seemed shorter than usual, as if their free ends had been in part dissolved away, increasing the diameter of the lumen of the gland. The deep staining in these glands was due to the ovoid cells, the central cells retaining the clear appearance common in hungering glands, and not becoming granular and readily stained as in normal digestion.

The breaking down of the central cells seems to be gradual, and none of the phenomena here described are seen simultaneously in all the glands of any one stomach. Sections shew some glands with normal hungering central cells; others appear to have their outer portions more or less broken down and gone; while in others the central cells are entirely wanting. That the central cells in this latter case have not been mechanically broken away by the stimulus employed seems to be satisfactorily shewn by the fact that they may be wanting in parts where the surface and vestibular epithelium is quite intact. This and the orderly way in which the cells disappear from without inwards seem to indicate that the process is a normal secretive one, however magnified by the unnatural conditions to which the animals were exposed.

If the central cells do in this way disappear in the course of normal secretion, there must be some means for their replacement, and sections from the above stomachs seem to indicate that they take their origin from the ovoid cells, usually by division, perhaps sometimes by direct metamorphosis. Signs of fission among the ovoid cells are numerous.

In most of the stomachs experimented upon, many of them are unusually large, and commonly contain two, sometimes three nuclei, these appearances being most common at the deeper parts of the glands (Fig. 7, c). Cells are also seen with an apparent division line across the centre and a nucleus in each half. In a single gland gradations may be found from a large, usually elongated, 'ovoid' cell with two or more nuclei, to small closely packed cuboidal cells with single nuclei, the intermediate forms being describable as large cells just about to divide, or small ones ill defined. In Fig. 7, at *d*, forms are seen combining the characters of both 'ovoid' and central cells; in Fig. 10, at *c*, is an appearance as if division had just taken place. When the central cells are absent their place is frequently occupied by elongated 'ovoid' cells containing more than one nucleus, and which by division would give cells somewhat of the form and in the place of the missing central cells. The immediate result of the division is not a typical central cell; it is smaller and stains more darkly, and from the nature of the experiment we might expect that these cells would go more slowly than usual through the internal changes necessary for the completion of their structure.

I am not prepared to maintain that division of an 'ovoid' cell is a necessary preliminary to the formation of a central cell; sometimes appearances are seen which suggest a direct transformation, and often it is impossible to say definitely to which group a given cell belongs. Staining specimens from these stomachs with osmic acid brought into view 'ovoid' cells, some stained as darkly as is usual with this re-agent, while others remained as light a hue as central cells commonly do. Close observation of normal stomachs shews in less degree similar appearances: central cells, for instance, are frequently stained on their deeper sides by aniline blue, quite like 'ovoid' cells, and 'ovoid' cells with two nuclei can be, here and there, almost always found.

I believe the difference between the normal stomach and the stomachs of long-starved animals treated as above described, depends upon the different rate and extent of cell metamorphosis in the two cases. Normal secretion is regular and probably occupies the glands by piecemeal, and the cells of any secreting part are replaced, step by step, as they are broken down: while in my animals not only was the stimulus abnormally prolonged and powerful, but the previous starvation of the animal, and the absence of food-materials which could be absorbed from the stomach during the stimulation, made the cell regeneration slow and deficient. Herrendörfer", so far as I know, was the first

to maintain a genetic relationship between the 'ovoid' and central cells. He supposes that the 'ovoid' cell secretes, and in the process shrivels up into the central cell. I have already given reasons for believing that the 'ovoid' cell is not concerned directly in secretion, and must therefore differ from him on that point.

If the fate of the 'ovoid' cells is to give rise to and replace central cells, there must be a source of new 'ovoid' cells, and that outside the gland itself: the following observations bear up this point. The corpuscles in the mesoderm beneath the mucous membrane of the stomach are commonly much like ordinary connective-tissue corpuscles: they are more or less branched, and radiate or triangular in optical section. Sections from the stimulated starving stomachs shewed in addition undoubted 'ovoid' cells in considerable numbers in this mesoderm and entirely outside the glands. This is most marked in the deeper parts of the mucous membrane where the mesoderm is thickest, as at *b*, Fig. 7. These cells may abut directly upon the glands, or be isolated from them at different distances in the mesoderm: many of them are of the common 'ovoid' cell shape, but are larger, stain less deeply, and are not so sharply defined. Between such cells and the typical mesoderm cell may be found all intermediate forms, some of which may be seen at *a* in Fig. 7. Staining with osmic acid makes the undoubted connective-tissue corpuscles very dark, especially the smaller and more branched forms. In the normal stomach also cells are frequently found which have the position in the gland, and all the characters of typical 'ovoid' cells, except that they are more or less branched, their processes penetrating between the neighbouring central cell. I believe then that it is very probable that, in the adult, the mesodermic corpuscle is the antecedent of the 'ovoid' cell; in this way also the appearance of cells like 'ovoid' cells in the mesoblast of the embryonic stomach gains a significance, although I think it probable that the first 'ovoid' cells in the embryo are formed from the 'embryonic' gland cells of the hypoblast. Once, however, the original hypoblast cells have been completely differentiated into 'ovoid' or central cells, I believe that in future the new 'ovoid' cells are produced by differentiation of the surrounding mesoblast corpuscles, which still retain their embryonic powers and characters; and that from the 'ovoid' cells so produced new central cells are formed to replace those broken down in the process of secretion. It is possible that the abundant nuclei of the adenoid tissue of the adult mucous membrane have some part in this process of cell renewal.

To sum up :—

The stomach glands are formed by ridge-like outgrowths from the surface of the mucous membrane. The hypoblastic cells, at first in a single layer, become several layers thick before the formation of the ridges, and become single again over these. The ridges are supported by mesoblastic outgrowths. By the intersection of the ridges pits are left which are the gland pouches. In these points I only differ in detail from Laskowsky and Brand.

The 'ovoid' cells are first specialised and in early embryos are found in the pyloric glands. The 'central' cells are specialised considerably later.

The stomach of the embryo sheep some time before birth possesses proteolytic and milk-curdling powers.

The central cells, to judge from the concurrence of the period of their well-marked specialisation and that of the first appearance of definite proteolytic properties in the mucous membrane, are probably alone directly concerned in the formation of pepsin (or a pepsin-zymogen).

The formation of pepsin (or the pepsin-zymogen) and of free acid in the stomach, are not parts of one and the same metabolism.

The cells of the mesoderm are probably the progenitors of all the cells in the gastric glands except those first formed in the embryo. They give rise to 'ovoid' cells, which in turn by fission (and perhaps by direct metamorphosis) form central cells.

#### DESCRIPTION OF PLATE XII.

Fig. 1. Intestine, sheep embryo  $\frac{1}{12}$  inch long. Transverse section.  $\times 380$  diameters.

Fig. 2. Stomach, sheep embryo  $\frac{5}{8}$  inch long. *a*, cells of hypoblast; *b*, cells of mesoderm.  $\times 380$  diameters.

Fig. 3. Flap and gland processes from sheep embryo  $4\frac{1}{2}$  inches long. *a*, gland processes; *b*, mesoderm; *c*, developing muscle fibres.  $\times 220$  diameters.

Fig. 4. A single gland process from the same, more magnified. *a*, flattened layer of mesoderm cells.  $\times 380$  diameters.

Fig. 5. Sheep's stomach near cardia. Embryo 8 inches. *a*, gland processes; *b*, ovoid cells, somewhat too sharply defined; *c*, doubtful forms; *d*, central cells; *f*, embryonic cells; *g*, blood-vessel.  $\times 380$  diameters.

Fig. 6. Section from pyloric part of same. *a*, embryonic cell; *b*, ovoid cells.  $\times 380$  diameters.

Fig. 7. Stomach (cat) near cardia. Shews gradations between mesodermic corpuscle and ovoid cell, *a*; ovoid cell in mesoderm, *b*; with double nucleus, *c*; intermediate forms, *d*; gland without ovoid cells, *f*.  $\times 380$  diameters.

Fig. 8. Pylorus (?) from fasting cat after stimulation. *a*, ovoid cell.

Fig. 9. Surface of mucous membrane from fasting stimulated cat. *a*, epithelium; *b*, ovoid cells.

Fig. 10. Vestibule of gland from cat as above (osmic acid). *a*, epithelium; *b*, ovoid cell; *c*, transformation form.

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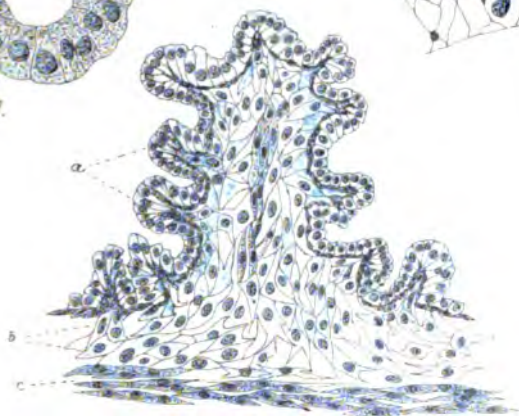




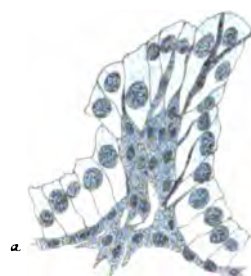
*Fig. 1.*



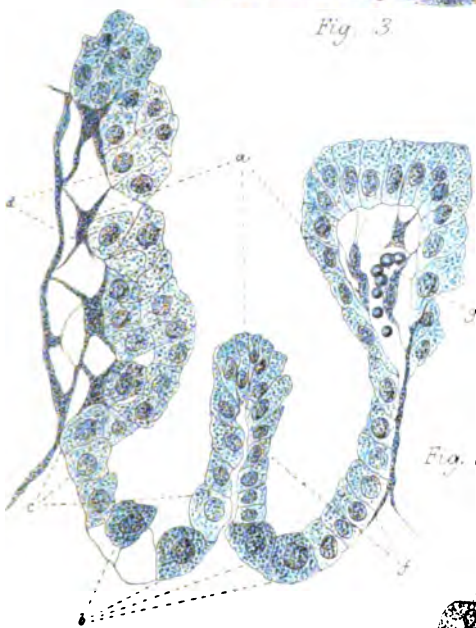
*Fig. 2.*



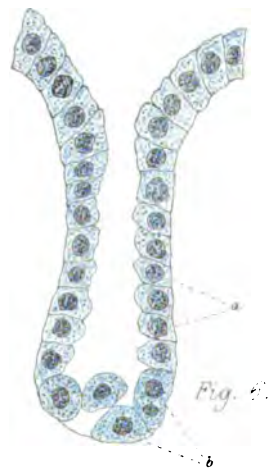
*Fig. 3.*



*Fig. 4.*



*Fig. 5.*



*Fig. 6.*



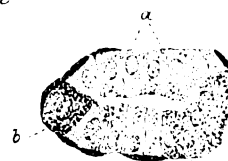
*Fig. 8.*



*Fig. 7.*



*Fig. 9.*



*Fig. 10.*



[*From the Journal of Physiology*, Vol. I. Nos. 4 and 5.]

**THE INFLUENCE OF STIMULATION OF THE MID-BRAIN UPON THE RESPIRATORY RHYTHM OF THE MAMMAL.** By H. NEWELL MARTIN, M.A. D.Sc.,  
*Professor of Biology, Johns Hopkins University, Baltimore, U.S.,* and  
W. D. BOOKER, M.D. (With Plate XIV.)

ONE of us having found (*Journ. of Physiol.*, Vol. I. p. 131) that chemical stimulation of the midbrain of the frog causes an alteration in its mode of breathing characterised by accelerated or tetanic inspiratory and impeded expiratory movements, the following experiments were undertaken with the object of ascertaining if the same phenomena were exhibited by the mammal. In the paper above referred to (p. 170) a bibliography is given of observations previously made as to the effects of cerebral stimulation upon respiratory movements; we have seen nothing since which calls for addition to that list.

Our first attempts at recording the rate and extent of the respiratory movements were made either by means of a flat inflated sac, connected with a recording tambour, introduced between the liver and the diaphragm through an incision in the *linea alba*; or by direct observation of the diaphragm exposed from the abdominal side. Neither of these methods was satisfactory, and we therefore had recourse to the following. Tracheotomy having been performed, a glass T-piece was tied in the windpipe: one limb of the T-piece had attached to it a short piece of india-rubber tubing: from the other limb a tube led, through a hole in the cork, into a large glass jar: through the cork of this jar passed another tube which was connected with a Marey's recording tambour, the lever of which wrote on a revolving cylinder. Near the bottom of the jar was an aperture which could be opened at pleasure, so as to set its cavity in communication with the surrounding air in the intervals of the observations. In these intervals the short india-rubber tube above mentioned was left open, and served for the animal to breathe through: when an observation was to be made this tube was closed by pressing between the thumb and finger or by a screw clamp, and the animal then breathed into and out of the glass jar: the variations of pressure produced in the latter were recorded by the tambour, which was inverted. Consequently, when the animal's chest

expanded (*i. e.* during inspiration) air passed into it from the jar and tambour, and the lever of the tambour ascended; the reverse of course occurred during expiration. By this means we found that very small respiratory changes in the capacity of the chest were very distinctly recorded while, as we satisfied ourselves by several experiments, the size of the jar was such that no dyspnoea of importance was caused by the animal's being compelled to respire from it for at least a minute. Fig. 1, Pl. XIV., shews a tracing, taken without any stimulation, for more than 90 seconds. An explanation of it will serve to elucidate the other figures also. The small movements at *a* are those traced by the tambour lever before the closure of the short india-rubber tube on one limb of the T-piece. On screwing up the clamp closing this, the respiratory tracings increase in extent and rapidly attain a maximum, which is then maintained nearly constant, both as regards their amplitude and rate, until *b*, when the short tube was opened again: there is no sign whatever of that tetanic fusion of the respirations, which, as will be seen subsequently, follows the midbrain stimulation. The line *x* is the base line traced by the tambour lever after we had disconnected the glass jar from the trachea and placed it in communication with the exterior air, while the drum made a second revolution. The line *s* was traced by the stimulation lever, to be hereafter mentioned. The time is indicated on the line *t*, traced by an electric chronograph in connection with a seconds clock. In the respiratory tracing there is a slight indication of a phenomenon, which in two or three cases occurred to a large extent and rendered the experiment useless, *viz.*, a larger absorption of gas by the lungs than was compensated for by exhalation, so that the total volume of gas in the apparatus was diminished, and the lever-tracing left the base line entirely. In the great majority of cases, however, this is entirely absent, or occurs to a very slight amount.

Rabbits were the animals employed throughout, except a couple of confirmatory observations on cats. After the insertion of the tube in the trachea, the general course of the experiment was as follows. The animal, still under ether, was turned over and the skull opened over the midbrain. The transverse sinus, which lies over the posterior tubercula quadrigemina, was thus exposed. If the posterior tubercula were those which it was desired to stimulate, a pair of needle electrodes, covered with a thin layer of sealing-wax, except for  $\frac{1}{8}$  inch at the tip, were inserted; this we were generally able to do without penetrating the sinus: when the latter was pricked we usually succeeded in stopping the bleeding by tying the sinus carefully on each side of the wound.

The electrodes were borne by a wire framework attached to the head holder of Czermak's rabbit bed, and so followed the movements of the head of the animal if it jerked it; in this way any tearing of the brain, which at first gave us much trouble, was avoided. If, on the other hand, it was desired to insert the electrodes into the anterior tubercula quadrigemina, either the posterior parts of the cerebral hemispheres were first removed, or, more frequently, the whole of them, as we found the latter operation, as a rule, caused less bleeding.

The electrodes were connected with the secondary coil of a du Bois induction apparatus, with magnetic interruptor; the current from a single carbon-bichromate cell was sent through the primary coil. In the secondary circuit was interposed the stimulation lever above referred to, which marked on the drum in the usual manner, when the current was sent through the electrodes. With reference to this marking, one point may require notice. At the commencement of an observation all three levers, time, stimulation, and respiratory, were placed in contact with the drum and on the same vertical line, the respiration lever being in that position (base line) which it assumed when the jar, with which the tambour was connected, was disconnected from the trachea, and in free communication with the external air. When the jar was closed and connected with the trachea the respiration lever described large curves, and was no longer on the same vertical line as the other levers, except at the moments when its tracing cut the base line. Consequently, to find at any moment what part of the stimulation line corresponds to a given part of the respiratory tracing, it is necessary to draw a perpendicular from the stimulation line at that point to the base line, and from the point where it meets the latter to draw a line parallel to the curves of the respiratory tracing until it meets the latter: this point of meeting will be the point in the respiratory curve answering in time to the given point in the stimulation line. The same is of course true for the relationships of the time and respiratory tracings. In fig. 6, *e.g.*, where the stimulation has lasted for 9 seconds (from  $s'$  to  $s''$ ), the point of the respiratory trace which answers to the moment of cessation of the stimulus is not that at which the perpendicular  $px'$  cuts the respiratory tracing; but is to be found by drawing from  $x'$  the dotted line  $x'z$  until it cuts the respiratory tracing. The point of the respiratory trace, which answers in time to the commencement of the stimulation, is of course to be found similarly. These dotted curves have not been drawn for the other figures in most cases, but the same reasoning applies to all.

After the completion of the operation the animal was left for some time, usually an hour, to recover from the shock of the operation. The midbrain was then stimulated during a minute and a half, in the manner above described, generally for periods of 10—12 seconds at a time, with intervals of 10 seconds rest. An interval of 10—15 minutes was then allowed to elapse, and the stimulation repeated with varying positions of the secondary coil. During the stimulation the etherisation was not pushed to the most complete narcosis, as otherwise inconveniently strong currents were required to produce any effect.

The general result of our experiments may be summed up thus: there lies deep in the midbrain of the rabbit, beneath the posterior corpora quadrigemina and close to the *iter*, a respiration-regulating centre, similar to that in the corpora bigemina of the frog: electrical stimulation of this centre causes accelerated inspirations finally passing into tetanic fixation of the chest in an inspiratory condition: and correspondingly diminishes or altogether inhibits expiration. If the animal be young and the operation have been performed without loss of blood, these results follow extremely feeble stimulation: stimulation, for example, with the secondary coil at 20 or 22, so feeble as to be barely perceptible when the electrodes are applied to the tongue.

Figures 2, 3, and 4 give the results of one experiment (Rabbit, April 25, 1878). The posterior part of the cerebrum was exposed at 11 a.m. with hardly any loss of blood: the electrodes were inserted at 12.15 p.m., and, as ascertained by *post-mortem* examination, the right electrode rather superficially in the right posterior corpus quadrigeminum and the left deep in the corresponding body on the left side. Fig. 2 gives the effects of four stimulations at 12.20. In the figure the lines *tt*, *ss*, and *x* are as in Fig. 1. The numbers beneath the stimulations give the position of the secondary coil during each. It will be seen that with the secondary coil at 20 the chest passed into a marked inspiratory condition, and never returned to its normal expiratory state during the stimulation. The inspiration is not however tetanic, but there are feeble attempts to return to the expiratory state, interrupted almost immediately by a fresh inspiration; we have in fact a number of very feeble rapid inspirations, which have the effect on the whole of keeping the chest fixed in an inspiratory condition. With the secondary coil at 22 this effect is still well marked; at 24 much less so, and at 25 it is only just recognisable. Fig. 3 shews the results of three stimulations at 12.30, with the secondary coil at 19, 18 and 17 respec-

tively; the tracings are of the same character as in Figure 2, but the influence of the stimulation is even more marked, especially that with the secondary coil at 17. Fig. 4 gives the results of stimulations commencing at 12.45, with the secondary coil at 16, 15 and 14 respectively: the last it will be seen passes into a continued inspiratory tetanus. Between each of these latter three stimulations a pause of two minutes was allowed, during which the animal was permitted to breathe freely. An attempt to get a more marked inspiratory tetanus by stronger stimulation failed (Fig. 5), the chest becoming relaxed during the stimulation: to this phenomenon we will return presently.

Figure 6 (Rabbit, March 29, 1878) shews a more perfect inspiratory tetanus, which is interesting from the resemblance of some of its features to those of an ordinary muscle tetanus; as for instance in the stimulation being at first insufficient to cause complete fusion of the discharges, but afterwards becoming so, as is the case in a muscle when fatigue comes on. On the other hand, in the mode of disappearance of the tetanic effect on the cessation of the stimulus, at first slowly and then more rapidly, we have a marked difference from a muscular tetanus. In this case the secondary coil was at 10.

We have numerous tracings resembling those just described, but it would be wearisome to give them in detail as they offer no essential points of difference. The reasons which cause us to locate the respiration-regulating centre in the precise locality above mentioned are as follows. When both electrodes are placed superficially in the corpora quadrigemina no respiratory effects follow, even with tolerably strong currents. On the other hand, when either one or both are pushed down to the level of the *iter* the results always follow (except when there has been much bleeding or when the animal is deeply narcotised), whether they be in the anterior or posterior tubercula; but the results are purer and obtained with weakest currents when the electrodes are in the posterior. When well placed in the latter very marked and characteristic effects can be obtained with extremely feeble currents, and they are then unaccompanied by any other disturbances. If the current is a little stronger general slight tremor of the whole body accompanies the respiratory effects. This tremor, which is so common, an expression of terror, is interesting in connection with Ferrier's view that the mid-brain mainly is concerned in the expression of the emotions. With powerful currents general clonic spasms, passing into general tetanus, come on. When the stimulus is applied to the deep parts of the anterior tubercles the tremor is usually absent, but the stimulation is



often accompanied with violent rhythmic efforts to draw the head back; sometimes also with twitching of the ears.

The extreme feebleness of the currents which give characteristic results seems to preclude the possibility of the stimulation of neighbouring parts by radiation of the currents being the cause of the phenomena. We made however several experiments with the view of excluding this possible source of error. Perfectly typical results were obtained after removal of the cerebral hemispheres and of the optic thalami and corpora striata (the latter in the cat), so that the phenomena are not due to fear or pain (which were also eliminated by the ether), or to the radiation of currents to any of those parts. Removal of the cerebellum also did not prevent the occurrence of the phenomena: after all these operations however somewhat stronger stimulation was required, doubtless on account of the diminished irritability resulting from the more severe operation. Finally we endeavoured to eliminate any result due to escape of the current to the medulla oblongata by severing the mid-brain from the parts behind after finding the normal results, and then trying again the effect of the midbrain stimulation. By the severance electric continuity would not be impaired, and any part of the effect due to radiation of the current to the medulla might still be expected after it. To make the division complete without injuring the midbrain was we found no easy task, but in two cases in which *post-mortem* examination shewed that we had accomplished it, and after the animal had been allowed an hour to recover from the shock, no results followed the midbrain stimulation until the secondary coil was pushed up over the primary, giving currents of such strength that, as evinced by the contraction of the muscles in the neck, &c., they escaped in all directions. Then the chest suddenly passed into an inspiratory state, but with no signs of accelerated inspirations; the inspiratory muscles were obviously merely held in tetanic contraction by the current reaching directly the respiratory tracts in the spinal cord or medulla. Stimulation of the medulla directly by the insertion of the electrodes into it produced similar results, with general tetanus. We believe therefore that we are justified in ascribing the effects observed by us, to the stimulation of a respiration-regulating centre lying deep in the back part of the midbrain.

We had not expected to find as marked expiratory effects in the rabbit, where the expirations are mainly passive, as in the frog where they are due to active muscular contractions; and during the more or less tetanic inspiratory conditions represented in Figures 2, 3, 4, 5 and



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## THE BOTANICAL RELATIONS<sup>1</sup> OF TRICHO- PHYTON TONSURANS.

By I. EDMONDSON ATKINSON, M. D.,  
OF BALTIMORE.

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THE results obtained by those who have made the life histories of the fungous growths, which are the exciting causes of certain diseases of the skin of man and some other mammals, the subject of study, have differed so widely that one is impelled to adopt one of two conclusions: either there exists in these forms of vegetable life a polymorphism exceeding the most extravagant claims of Hallier, or else the methods of investigation adopted by these observers have been sadly inaccurate and exposed to all sorts of adventitious influences. With the best mycologists of the day the opinion prevails that, while a limited polymorphism may be admitted, one may reject, without hesitation, those theories which would embrace in one genetic series the different fungi to which the diseases under consideration have been attributed. On the other hand, a moment's reflection must make it evident that the methods of cultivation employed have been open to the gravest objections, and the cultivations themselves exposed to the most varied contaminations. Results obtained from cul-

<sup>1</sup> Read before the American Dermatological Association, at Saratoga, August 28, 1878.

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tivation upon such nutritive soils as sliced vegetables, potatoes, carrots, apples, and the like, must prove absolutely untrustworthy, since it is a matter of every-day experience that all such preparations will inevitably become the hosts of multitudes of spores, which rapidly invade, conceal, and overwhelm the less vigorous spores planted upon them. No accurate worker would, to-day, dream of obtaining truthful results through such unscientific methods.

A much more reliable process than the foregoing has been used by certain mycologists, who claim a high degree of purity for the cultivations conducted by it. According to Brefeld, this method consists in sowing a single spore in a drop of a given nutritive fluid, upon a glass slide resting upon a metallic plate, under a bell-glass, and protected from external influences by having the latter resting in water. (*"Botanische Untersuchungen über Schimmelpilze,"* Heft 1, p. 5). Minute precautions should be used to thoroughly purify all the materials employed. This plan offers manifest advantages over the previous one, but the liability to contamination still remains excessive; for, not only is it impossible to secure the necessary purity of the atmosphere of the bell-glass, but the frequent removals of the latter for purposes of inspection and of supplying the losses of nutrient fluid by evaporation must inevitably lead to the lodgment of adventitious spores of yeast, mould and bacteria, and the like, circumstances, when not fatally interfering with the growth of the special fungus, affording great danger of confounding the latter with intruders of widely different nature.

Desiring to make some study of these parasitic fungi, and being conscious of the imperfections of the foregoing methods, I fortunately made known my difficulties to Prof. H. Newell Martin (in whose laboratory, at the Johns Hopkins University, the more important of my researches have been followed), who suggested to me the method employed by MM. Van Tieghem and Le Monnier in their observations upon the *Mucorini*, published in the 17th volume of the *"Annales des Sciences Naturelles"* (pp. 261-399). His plan may be called the cell culture, and, briefly described, is as follows: The cell is constructed by fastening with Canada balsam, upon a glass

slide, a glass ring from four to five millimetres in height, and about fifteen millimetres in diameter. It should be ground flat upon its edges. A thin cover-glass, as thin as can be procured, of the diameter of the ring, forms the roof of the cell. When it is to be used, a drop of nutritive fluid is placed upon the cover-glass, and into this drop the fungus is sown. The cover-glass is then placed upon the ring, with the drop upon its under-surface, a drop of boiled distilled water having been previously placed in the bottom of the cell, to secure the proper atmospheric moisture. The cover-glass is kept in position and protected from the external air by a few minute drops of oil. In pursuing this method it is, of course, necessary to observe all possible precautions to prevent the introduction of foreign spores. The nutritive fluid, the distilled water, and the oil, should be boiled in test-tubes, stoppered with cotton wool and only opened at the instant of using. I have adopted the plan of drawing these fluids into fine pipettes previously subjected to an extreme heat. In this way a drop of the required minuteness can be obtained quite uncontaminated. The cell and cover-glass must be scrupulously clean and all accessory apparatus thoroughly purified. When finished, the cells should be placed side by side in a box, half filled with moist sand, and protected by a lid or a piece of glass. In the winter it will be advisable to keep the box in a water bath at a temperature of from 20° C. to 30° C., or upon a mantel-piece over a fire. In summer no such precautions are necessary.

The cell may now be examined under the microscope, and every part of the drop observed. With thin cover-glasses quite powerful objectives can be used. In my investigations a Zeiss's D objective was most conveniently employed, although it was possible to use with profit the F objective of the same maker. The advantage of the greater amplification of the latter objective, however, was more than counterbalanced by the danger of breaking the cover-glass in obeying the almost irresistible impulse to peer as deeply as possible into the cell. One of Grunow's  $\frac{1}{8}$ " objectives of 110° angular aperture was also easily used. With the D objective and No. 4 eyepiece, an amplification of 400 diameters was attained.

I have employed as nutrient fluids Pasteur's fluid, with and without sugar, distilled water, orange-juice, decoction of horse-dung, aqueous humor, gelatine, currant-jelly, and meat infusion. Of these, orange-juice has seemed the most suitable, although I have succeeded with Pasteur's fluids. The horse-dung decoction, so highly recommended by Brefeld, I have found so extravagantly disposed to the development of bacteria that it has been useless in my observations. The acidulated solutions were preferable, on account of their freedom from bacteria.

It must at once be admitted that it is impossible for strange germs to find their way into a cell after its completion under the above-mentioned precautions, unless it be by thrusting their hyphae between the cover-glass and cell, a proceeding that can easily be detected. It remains to be seen to what extent the culture can be kept pure during the moments occupied in the preparation of the cell. It shall be my endeavor to show this later.

The fungus I selected for cultivation was "*Trichophyton tonsurans*," taken at different times from the heads of two light-haired boys. After thoroughly washing the affected surfaces, I extracted very short stumps of hairs, with as much of the bulb, or lower part of the shaft, as possible, this being a procedure of much difficulty, since in a large majority of cases the hairs break off outside of the follicular orifices. I selected portions of hairs rather than single spores, partly because it has been with me the rarest occurrence to see a spore sprouting apart from its habitat, the hair; partly on account of the infinitely slender chances of selecting a spore capable of budding in cell cultivation; but chiefly, because the style of germination in a successful cultivation has been so distinctively characteristic, that I have considered the results obtained sufficiently convincing.

It must not be supposed, however, that germination occurs readily in these cells. On the contrary, probably on account of the restricted air-supply, it is, by far, the usual experience to find the cell remain absolutely quiescent, the homogeneous and apparently perfect spores remaining for weeks unchanged, finally to slowly disintegrate. As Van

Tieghem and Le Monnier have remarked, the causes of failure in cell cultures are very different, and by no means obvious. This much is certain: that a large proportion of cells, with hairs full of spores in apparently perfect condition and remaining entirely free from adventitious growth, and kept under observation for many days, show not the smallest sign of development. This indisposition to germinate is not common to all fungous forms when sown in cells, as is proved by the facility with which penicillium and aspergillus shoot out their vigorous hyphæ, when their spores have accidentally or designedly been introduced; and especially by the success with which MM. Van Tieghem and Le Monnier cultivated the various forms of mucor, obtaining even the sexual reproductive process, a development that Brefeld has never observed in fluid cultivations.

Where, however, a successful cultivation is secured, never does a single nor even do a few hyphæ appear; but there is invariably a multitudinous and simultaneous outburst of growth of hundreds of spores, indicating that the conditions of life and development depend upon some special appropriateness of the cell and of the fungus.

The history of a successful cultivation, then, is as follows:

A short, broken hair, extracted with as much as possible of the part within the follicle, containing the more active spores, is secured, and with all practicable dispatch is sown in the nutrient fluid, and the cell completed by laying on the cover-glass. In from twenty-four to thirty-six hours, more usually the latter period, but frequently only after several days, signs of vigorous growth will become evident. The spore mass will be seen to exceed its boundaries of the day before, projecting in a single or double rows or more beyond the hair, both in the direction of its axis and laterally. These spores, whose nutriment has been abundantly supplied, will sometimes be observed to swell to many times their original proportions, attaining sometimes a diameter of .0222 millimetre. (*See Fig. 2, b.*) This process is not often observed in its fullest degree, and ceases as soon as hyphæ begin to be freely thrown out. I have been unable to decide whether the insignificant increase in the area occupied by the spore mass is due

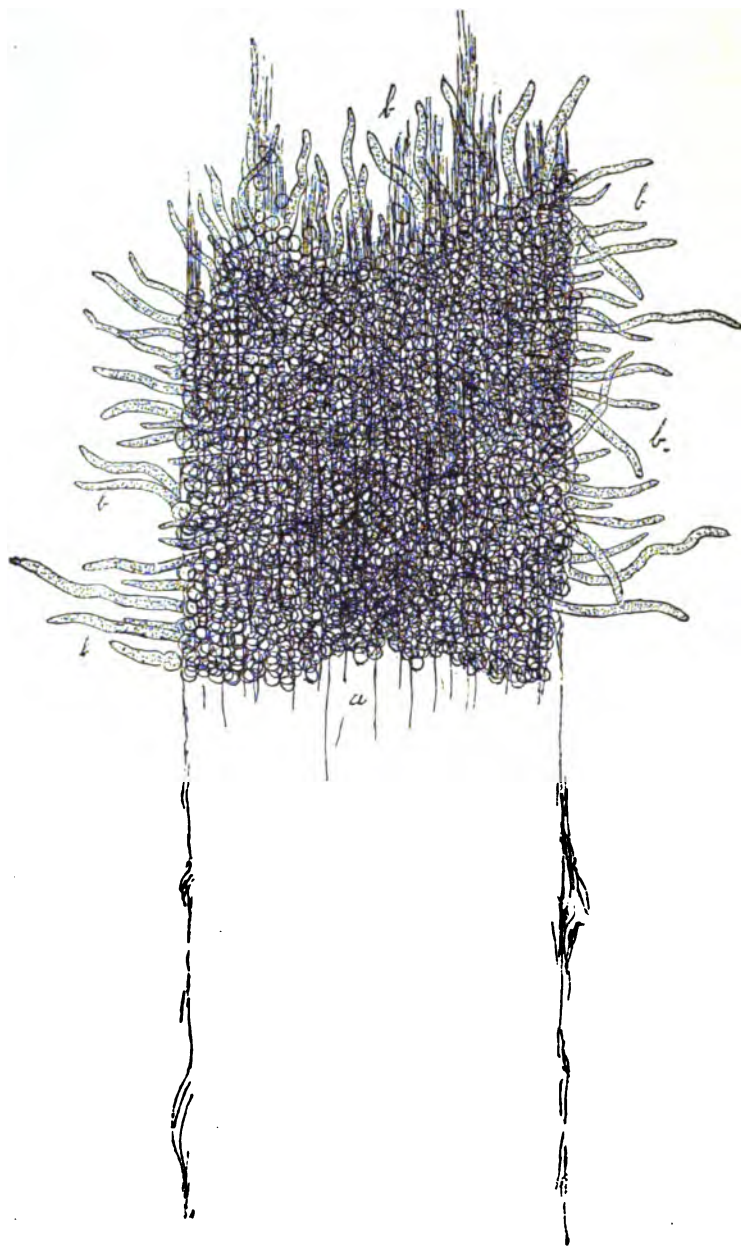


FIG. 1.



to this swelling alone, or to an actual increase in the number of spores by a simple budding, torula-like process, as well. I have not detected the latter in cultivation, but am satisfied that it occurs, since in the growth of the fungus in the hair, just after detachment, many partially budded spores are visible, but their growth seems to be arrested by their transference to the culture fluid. Spores which have swollen to the larger diameter undergo no further development, or throw out short hyphæ, which remain unbranched and whose growth soon becomes arrested. Almost simultaneously with this swelling of particular spores, or even, perhaps, without this having occurred, hyphæ may be seen shooting out from the hair-shaft in hundreds (Fig. 1, *b*), the spores from which they spring, as well as those which have undergone no change, having a diameter varying from .002 millimetre to .005 millimetre for the globular ones; and for the oval or oblong ones from .004 millimetre to .0045 millimetre in breadth, and from .007 millimetre to .01 in length. The hyphæ have at the same time an average diameter of .0025 millimetre or more, and grow, as yet, without dividing and without forming septa. They spring, medusa-like, from the hair, and may occasionally be traced to their proper spores, which may begin to be slightly vacuolated.

The abundance of nutriment being favorable to the formation of a mycelium, the hyphæ now freely branch, and by the third day many have become septate, the segments becoming frequently irregularly bulbous or forming globular swellings (see Fig. 2) of very much increased size, .015 of a millimetre or more in diameter. These conditions may be observed exhibited in Fig. 2. By this time, the mycelium begins to form a network of greater or less density, and already at numerous points, both lateral and terminal, short hyphæ have been thrown out, bearing at their terminations globose bodies with granular contents, occasionally vacuolated, and which quickly become separated from the hyphæ by partitions directly transverse to the hyphæ and presenting sporangial characters (see Fig. 2, *d*). Most of these sporangium-bearing hyphæ are devoid of septa until the formation of the one representing the columella, but the mycelium from which they arise possesses septa at tolerably

wide intervals (*see* Fig. 2). By about the fifth day the hyphæ and mycelium become freely vacuolated and the sporangia begin to exhibit little aggregations of protoplasm, the future

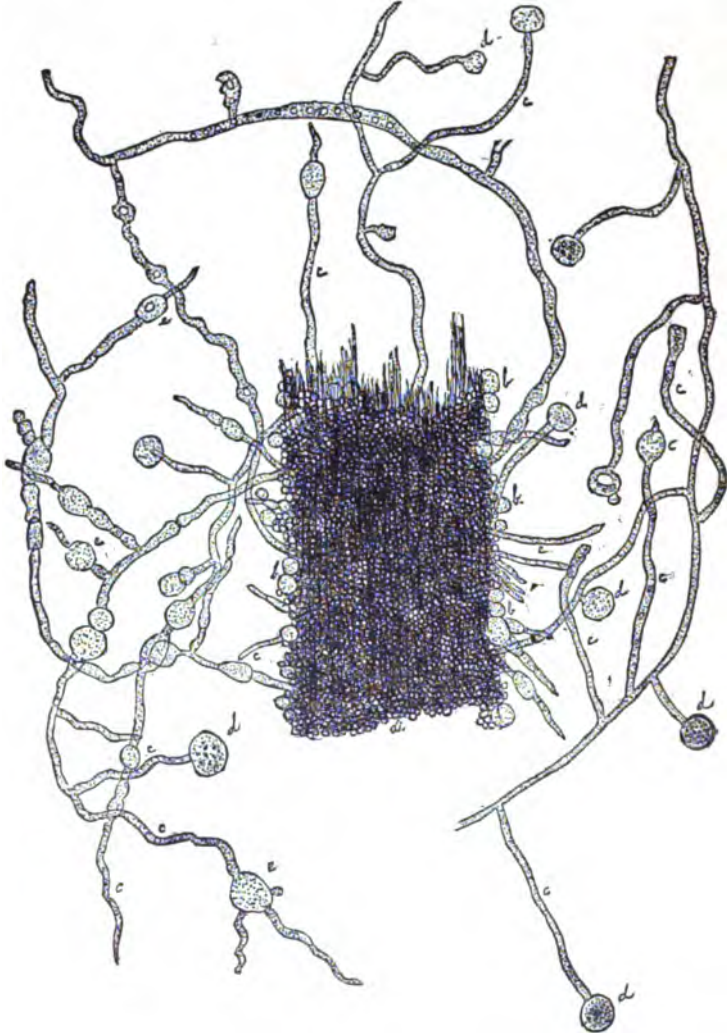


FIG. 2.

spores. The septa between them, however, and the hyphæ remain quite flat. These sporangia may attain an extreme

diameter of .018 millimetre. Here and there the hyphæ show a tendency to break up into small segments (*see* Fig. 3, B), a process, however, which I have never seen carried to the extremity of spore or brood-cell formation. Occasionally, also, instead of retaining their sporangial characters, the sporangia, as if diverted from their original purpose, develop buds at one or more points of their surfaces, which in their turn become hyphæ. (*See* Figs. 2 and 3, *c.*)

With abundance of nutriment, the tendency of the hyphæ to grow into a closely meshed mycelium is very marked, and but comparatively few distinctive reproductive organs are developed. It is in the underfed cultivations, in those where the actively growing hyphæ soon exhaust the fluid surrounding them and where the remaining vigor is diverted to the formation of reproductive organs, that the growth is followed with most facility and most decided results. In such a cell, while the swelling of the spores is less noticeable, the hyphæ are thrown out precisely as when more abundantly nourished, and for a limited period (thirty-six to forty-eight hours) pursue a similar course. Beyond this point they become less vigorous, their branching becomes arrested, and their advancing growth ceases. Septa usually, though not always, appear in the hyphæ, which sometimes shows bulbous irregularities, as if about to form jointed spores. About the third day some of the hyphæ show sporangium-like enlargements (Fig. 3, *d*), both terminal and lateral, in which vacuoles appear, and which exhibit no columellæ or partitions from the hyphæ, in which by this time vacuoles have become abundant. In instances most favorable to observation, the hyphæ spring directly into the formation of sporangia without a single branching and quite often without the formation of septa, and after very insignificant growth, just as the sexually produced zygospores habitually do. From this point their growth is somewhat indefinite and is evidently controlled by conditions of nutrition. A very few sporangia (Fig. 3, *g*) develop columellæ, but are apt to form no spores; others, with or without septa, form a few diminutive spores; others again, after forming sporangial enlargements, may develop buds which either grow as ordinary hyphæ or swell into sporangial forms, like beads strung to-

gether (Fig. 3, *e, f*; Fig. 2, *e*). In the very few instances where this reproductive process reaches completion, the wall of

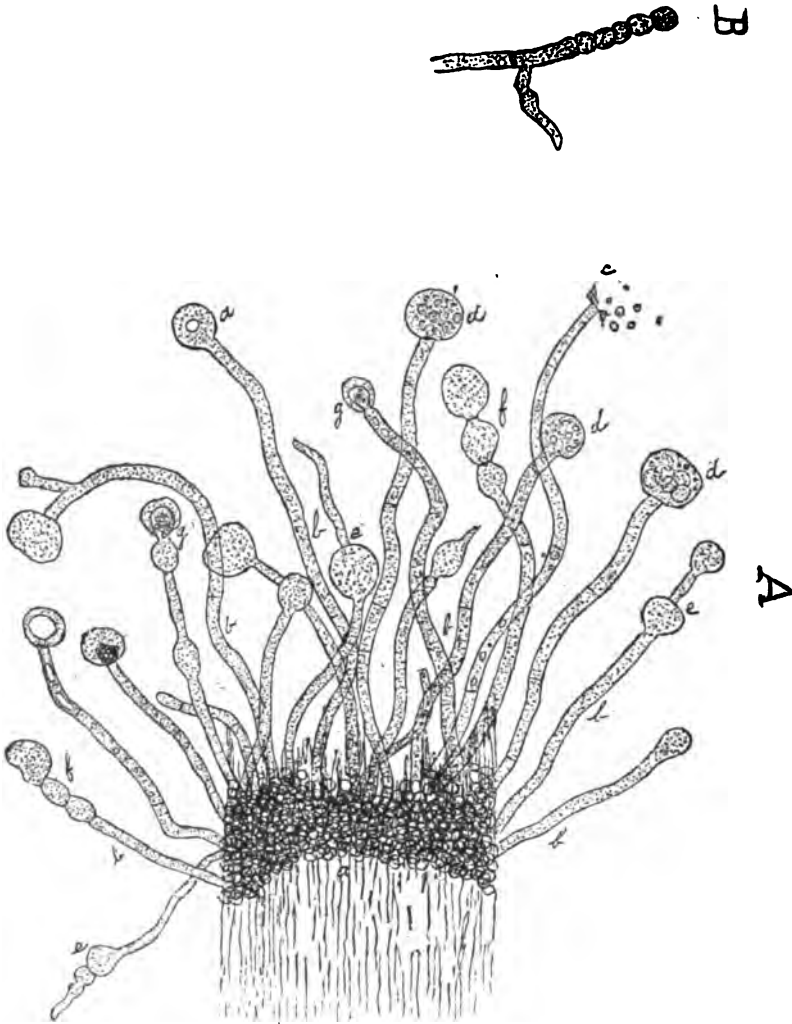


FIG. 3.

the sporangium bursts and four or five minute spores are released (Fig. 3, *c*). Here all signs of development are at an end, and the cell remains unchanged for an indefinite period.

The sporangia show by their varying forms that they have not acquired their full and perfect maturity, and that their development is controlled by the circumstances of their position, so that they betray a singular indecision, so to speak, in their final course.

My experience has not enabled me to speak positively concerning the formation of chlamydo-spores, or spores developed within the hyphæ, as described by Van Tieghem and Le Monnier, in their account of the mucors, nor have I detected any disposition toward the sexual union of hyphæ for the formation of zygosporoes.

A number of healthy hairs, removed from the heads of the children furnishing the fungus for my experiments, have been subjected to the same methods of cultivation, and have uniformly yielded a negative result.

Having thus followed the history of this fungus throughout its several stages, it will be proper, at this time, to ascertain the degree of confidence to be placed in the method of cultivation employed.

It is sufficiently evident that, after the completion of the cell, no contamination by foreign germs is to be feared. In the preparation of the cell, however, such accidents are liable to happen, and the unwelcome intruders are not slow to reveal themselves. The most frequent foreign germs are those of bacteria, which, however, are but little disposed to invade acidulated fluids. Penicillium, also, not unfrequently appears, but can always be traced, either by its hyphæ penetrating under the cover-glass, or radiating from a centre either in the surrounding fluid or at some point of the hair-shaft, and affording, in its style of growth, a striking contrast to the multitudinous, simultaneous outburst of activity from the hosts of trichophyton spores, along the whole shaft. In not a single instance where germination took place in the medusa-like manner I have described and figured, was there observed any other form of fructification than the sporangial one. Occasionally, during the growth of the hyphæ, a segmentation would occur indicating but not completing a breaking-up into torula-like chains of spores, beginning at the free end, the so-called brood-cell formation. Grawitz (*Virchow's Archiv*,

August, 1877) describes as derived from his slide and mass culture of *achorion Schönleinii*, *trichophyton tonsurans*, and *microsporon furfur*, a similar segmentation, and has concluded that these several forms are derivatives of *oidium lactis*, whose aerial fructification occurs in a somewhat similar centripetal segmentation. His drawings suggest, however, the torula-like brood-cell segmentations of other kinds of moulds.

It is of especial significance that, in cells whose conditions offer no obstacle to the development of ordinary mould fungus, the uniform result was constantly obtained, where the spores filling the hair germinated. And it is also of significance that, where this style of germination did not occur, the spores remained for weeks absolutely unchanged. It must be remarked, however, that the "*trichophyton*" does not germinate in cell culture with anything like the readiness of *penicillium*, *aspergillus*, or of ordinary *mucor*, or that itself displays in slide culture, where hyphæ are freely thrown out, but generally come to naught, on account of the unlimited growth of strange spores and bacteria. Indeed, although I have been able to follow in this slide culture the hyphal growth as far as the formation of sporangia, it has only been through my previous experience with cell culture that I have been able to distinguish the true parasite from the several other forms of fungus visible.

What the agencies are that prevent the free development of this fungus in cell cultivations, I am quite unable to say, although it is probable that the restricted supply of air has some influence. But it is a fact that successful results have been obtained in a very small minority of my cells, the vastly larger number remaining absolutely quiescent. This difficulty with which germination takes place is the only serious drawback to the method employed, which is one easily practised, readily available, guaranteeing the purity of the cultivation after the sowing; and, with scrupulous observance of all precautions in the preparations of the cells, their entire purity can be secured in a surprisingly large proportion. With a proper observance of details, and a patient persistence in the face of many failures, I am confident that my own observations will find confirmation at the hands of other investigators.

There remains, finally, the task of assigning the fungus of *tinea tonsurans* to its appropriate systematic position.

As has already been remarked, I have been unable to determine positively whether the increased area occupied by the spore mass, after growth has begun, is the result of the swelling of the spores, or of a positive spore increase through budding as well. I am, however, strongly impelled to adopt the latter opinion. But it must be borne in mind that this torula-like mode of growth does not imply more than a form of resemblance, and by no means the ferment-producing powers of yeast, and may be observed in a number of fungi (De Bary, "Morph. und Physiolog. der Pilze," pp. 119 and 182). At all events, in the cultivation of "trichophyton," this process ceases very early, as soon, indeed, as the hyphæ begin to grow freely.

In a hair invaded by "trichophyton" examined just after removal from the scalp, there will almost always be observed a decided tendency toward the division of the mycelium or hyphæ into very short segments, which bear every evidence of ultimately forming spores. This process, which I have frequently seen indicated in cell cultivation, but which I have never observed carried to completion, finds a perfect analogy in one of the reproductive processes of one of the mucors, *mucor mucedo*. De Bary says ("Morph. und Physiolog. der Pilze," p. 179) that in old mycelium, or in such as has, through deficient nourishment, deprivation of air, or other untoward influences, the formation of spores interfered with, short cylindrical sections filled with homogeneous protoplasm are formed by the appearance of septa, and become spores of a cylindrical, oval, or globular form. Doubtless, under more favorable conditions, this brood-cell formation is carried to its completion, in the development of *trichophyton tonsurans*; but for the present I must restrict myself to the statement that, in cell cultivations, this tendency is shown to be pretty constantly present. (See Fig. 3.)

In assigning "trichophyton" to the mucors, it will first be necessary to indicate some points in which the growth of the former differs from that usually ascribed to the latter. It will be observed that "trichophyton" departs from that feat-

ure characteristic of the family mucor, a unicellular, unsegmented condition of the hyphæ and mycelium previous to the formation of sporangia. This rule admits of some modification, however, since septa may appear when the protoplasmic contents become impoverished shortly before the sporangia begin to form. It must be remembered, moreover, that the formation of brood-cells requires a process of segmentation incompatible with an unvarying unicellular presporangial condition of the fungus, and depends usually upon unnatural and perverted influences. In slide cultures of "trichophyton," the hyphæ branch and attain a considerable length without the formation of septa; but these usually appear some time previous to the sporangia. In the cell cultivations, the septa appear earlier, although, where the hyphæ proceed immediately to the formation of sporangia, the septa may be absent.

Another point to be considered is the departure of this fungus from the type of sporangium development of mucor in the arrangement of the columella, which should project into the cavity of the sporangium in a conical shape. This may also, however, be regarded as a character subject to the altering effects of special influences. It will be observed that, of the sporangia represented in the drawings (Figs. 2 and 3), the greater number present only the straight septa dividing the sporangia from the hyphæ, others seeming to have been arrested in their growth before reaching this stage. Brefeld ("Botanische Untersuchungen über Schimmelpilze," Heft 11, p. 20) says that "sporangia starved or injured in their development or attacked by parasites vary greatly in size, and gradually lose the typical characters of mucor. The columella loses its shape, becomes smaller, and finally is entirely absent." The spores, according to Brefeld, may also diminish from their normal size, measuring sometimes as little as .0033 millimetre. It would seem, therefore, that the normal characters of mucor may be considerably altered by various disturbing influences; and, with the knowledge thus gained, it seems to me that a fungus presenting the features displayed in my cell cultivations may without hesitation be referred to the mucors.

In conclusion, I desire to express my sense of the imper-



fections of this paper, and my regret that I have been able to bring but a limited mycological experience to its preparation. I feel confident, however, that my observations as described and figured are correct, and that they will be confirmed by other investigators, employing the same methods of research.

The granular markings of the protoplasm in the illustration are rather too coarse.



**PRELIMINARY OBSERVATIONS UPON THE  
DEVELOPMENT OF THE MARINE PROSO-  
BRANCHIATE GASTEROPODS.** By W. K. BROOKS,  
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Hopkins University.*

THE segmentation of the egg among the Mollusca, and the early stages of development in the various groups, present so many variations that it is of the greatest importance that at least an outline of the process should be published for as many forms as possible.

During the summer of 1877 I made use of the opportunities which the laboratory of the United States Fish Commission at Wood's Hole afforded for studying the development of certain Marine Gasteropods, whose eggs are characterized by the presence of a large food yolk and the restriction of the segmentation to one pole of the egg. While my observations agreed in many points with those of Brobetsky, ("Studien über die embryonale Entwicklung der Gasteropoden," Arch. f. Mic. Anat., 1876,) my interpretation of the phenomena was totally opposite in certain important particulars. According to this observer, the mouth and foot are formed at that pole of the egg where the blastoderm finally closes together to surround the food yolk, and the definitive mouth is similar in position to the gastrula mouth. According to my observations, the mouth and foot appear upon that pole where the segmentation commences, and the gastrula mouth coincides, in position with the shell gland.

During the past summer, at Fort Wool, I reviewed the subject thoroughly and satisfied myself of the correctness of my conclusions, and I will, therefore, give a sketch of the more important points, illustrated by outlines from the finished drawings which may not be published for some time. As the paper is simply an abstract, all general questions, disputed points and comparisons with the results of other observers will be omitted. The drawings which are copied are from the eggs and young of two of our common Prosobranchs, *Astyris lunata* and *Urosalpinx cinereus*. Figures 5, 6, 7 and 8 are early stages in the development of *Astyris*, and all the others are the eggs and embryos of *Urosalpinx*.

The eggs of *Urosalpinx* are contained in small transparent membranous parchment-like vases, each of which is attached by an expanded foot to some solid substance, usually the under surface of an overhanging rock, a little above low tide mark. Each female deposits great numbers of these vases, from ten or twelve up to more than a hundred, and the process of laying occupies several weeks. When the animal is not disturbed during oviposition the vases are all attached, in more or less regular rows, to the same surface, and in this way an area of three or four square inches may be covered.

In shape and size they are much like the well known egg cases of *Purpura lapillus*, but they have not their slight red tinge of color. They are flattened vertically, and their edges are marked by keel-like ridges. Owing to the length of the period of oviposition, eggs and embryos in all stages of development are to be found in the various vases of a group, and the young escape from the first-laid vases before the female has finished laying. Unlike the vases of *Purpura*, each of which contains several hundred eggs, those of *Urosalpinx* contain only from six to twenty, ten or twelve being the usual number. All of these normally undergo development and give rise to embryos, although abnormal or retarded eggs are frequently found, and the rate of development varies greatly among the eggs in the same vase. Occasionally a partially segmented egg or a more advanced embryo becomes abortive and breaks up into separate cells, each of which remains alive for some time and often swims actively by the motion of its cilia. These "cosmellae" and the yolk of the aborted eggs are drawn into the digestive cavities of other embryos; but while this method of furnishing the young with food appears to be normal with *Purpura*, it is accidental and exceptional with *Urosalpinx*. The eggs are suspended in a tenacious transparent albuminous substance which at first fills the vase, but is used up as food by the growing embryo, which increases in size many hundred-fold before it leaves the capsule.

Before segmentation the eggs are nearly spherical, opaque, yellowish white, and are made up of a ground substance or network of transparent, very slightly granular protoplasm, the meshes of which are filled with highly refractive globules of deutoplasm or food material, which are packed into the protoplasm like the starch grains in a potato. The protoplasm stains readily with carmine or with osmic acid, and is then quite conspicuous. When an egg is torn to pieces with needles the spherules of deutoplasm fall out of the

spaces, but retain their shape, while the protoplasm still exhibits the sharply defined spherical chambers which contained them.

The animals will not lay their eggs in confinement, and as the vases must be collected, taken to the laboratory and opened before the eggs can be studied, the species is not a favorable one for studying the earliest changes, and its opacity prevents the internal changes which precede and accompany segmentation from being seen.

The first external change is the lengthening of one pole, so that the egg becomes pear-shaped. The portion thus drawn out now becomes less opaque than the remainder of the yolk, owing apparently to the absorption by the transparent protoplasm of some of the opaque food spherules, and we are now able to distinguish the formative from the nutritive pole. Two or three large spheres of segmentation now separate from the formative pole, as shown in Figure 1. As in many other mollusca, a well marked period of contraction succeeds each period of active segmentation, and the segmentation spherules are alternately sharply separated from the yolk, and then partially merged in it.

The spherules which first appear are less opaque than the food yolk, and one end of each of them, the end towards the left in Figure 1, is quite transparent.

We have then, at this early stage, one pole of the egg distinguished from the other by the presence of segmentation spherules; and one side of these spherules distinguished from the other by its transparency.

Since the subsequent history shows that the final mouth of the animal is formed at the nutritive pole of the egg, and that the opaque portions of the spherules are upon what is to become the dorsal surface, we may hereafter use the terms oral and aboral, dorsal and ventral. In Figure 1 the oral end is above, the aboral below, the dorsal surface to the right, and the ventral to the left. At the stage shown in Figure 1, a transparent area is visible on the ventral side of the oral end of the food yolk, *B*. It is probable, from Brobetsky's observations upon *Nassa*, that this is to be regarded as the point where a spherule, similar to those shown at *D*, has become fused with the food yolk. As the cells of the ectoderm are to be derived in great part from this transparent area, it may be called the *ectodermal area* of the food yolk. It stains readily with carmine or osmic acid, as do the transparent ends *D''* of the spherules *D*. A group of small transparent segmentation spherules now

makes its appearance at the oral end of the egg and ventral to the large spherules, and soon forms a distinct layer of ciliated cells: *the ectoderm*. This layer seems to be derived in part from the transparent ventral surfaces of the primary spherules or macromeres, but mainly from the transparent ectodermal area of the food yolk.

The macromeres *D* now divide and give rise to a number of opaque spherules, much larger than the ectoderm cells, and so arranged as to form a rim around the sides and dorsal edge of the ectoderm. As these opaque large spherules give rise to the wall of the digestive cavity, we shall call them the endoderm hereafter. Figure 2 is a ventral view, Figure 3 a dorsal view, and Figure 4 a dorso-ventral optical section of the oral portion of an egg at this stage, much more highly magnified than Figure 1. In Figure 2 the ectoderm is shown at *F* as a layer of small ciliated cells, bounded dorsally and at the sides by the endoderm spherules *D*, and ventrally by the food yolk *B*. The ventral margin *F'* of this layer now extends downwards onto the ventral surface of the food yolk, by the addition of new cells to the ventral margin *F'*. These new cells appear to be derived from the ectodermal area 1. The endoderm spherules *D* are seen at the sides of the layer of ectoderm, separating it from the food yolk, and also projecting above its dorsal edge. In the dorsal view, Figure 3, a small portion of the ectoderm *F* is seen above these spherules *D*. In the optical section, Figure 4, the ectoderm forms a single layer, *F*, of nucleated ciliated cells, which arch over a segmentation cavity *F''*. The letter *F'* indicates the ventral growing edge of the ectoderm, and 1 the ectodermal area. A comparison of Figures 2, 3 and 4 shows that the segmentation cavity is bounded below by the surface of the food yolk *B*; dorsally and at the sides by the macromeres *D* of the endoderm, and ventrally and orally by the ectoderm *F*. The latter now grows down onto the food yolk on all sides, and covers up the endoderm spherules and at the same time pushes them down towards the aboral pole. Figure 9 is the oral end of an egg at a somewhat later stage, in which the ectoderm forms a cap upon the oral end of the food yolk *B* and nearly covers the endoderm *D*. At this stage the embryo begins to rotate slowly by the action of the cilia of the cap of ectoderm. Figure 5 is a dorsal view of the entire embryo of *Astyrus lunata* at a somewhat later stage. The food yolk *B* is now nearly covered by the cap of ectoderm *F*, which also entirely covers the endoderm *D*. The foot is now present as a fold of ectoderm upon the oral ventral

edge of the embryo, and its corners are seen at *G*, projecting beyond the general outline of the oral surface.

The endoderm spherules *D* have not yet undergone very much change; they are covered by the ectoderm and are pushed down from their original position onto the sides of the food yolk, around the sides and dorsal surface of which they form an incomplete ring, open on the ventral surface, as seen in the side view, Figure 6. On the sides this belt is only one cell wide, but upon the dorsal median line it has begun to grow upwards towards the oral end of the embryo. The embryo shown in Figure 6 is also an *Astyris*, a little younger than that shown in Figure 5, and seen from the left side, or in the same position as Figures 1 and 4. Both figures are from embryos which had been placed for half an hour in one-fifth per cent. solution of perosmic acid, and had then been stained with carmine. The endoderm is so obscured by its covering of ectoderm that it cannot be satisfactorily studied in a living specimen, but it is quite distinct in stained specimens.

In this figure the projecting foot *G* is quite prominent, and as it is the first organ to appear and is present in both *Astyris* and *Urosalpinx* before the closure of the blastoderm around the food yolk is completed, as well as at all later stages, it is of the greatest importance in determining the relation of organs of later formation to the poles of the segmenting egg.

At *F'* on the ventral surface, under the foot, is the opening in the belt of endoderm, and over it the growing edge of the ectoderm. Figure 7 is a median vertical optical section of Figure 5, and Figure 8 a similar section of Figure 6. As the letters of reference are the same as in the previous figures, they will at once be understood from the previous description.

As development progresses, the sides and ventral margin of the ectoderm continue to extend down onto the food yolk, and at last surround it, meeting at a point which corresponds in position to the point *C* of Figures 5 and 6, which in its relation to the endoderm is the same as the point *C* of Figures 3 and 4. Meanwhile the oral half of the food yolk becomes absorbed, and the layer of endoderm grows upwards upon the sides and dorsal surface of the embryo and thus builds up a wall or parapet around three sides of the now nearly hemispherical food yolk, and this growth goes on until the upper margins of the walls meet at the oral end of the embryo. In this way the cavity which is left after the absorption of the food

yolk becomes arched over by a roof of endoderm concentric with the layer of ectoderm, but separated from it by the segmentation cavity.

The primitive digestive cavity which is thus formed is open along the ventral median line, and it is bounded below by the oral surface of the food yolk and dorsally and laterally by the endoderm. It opens externally by a "gastrula mouth," the position and mode of formation of which will be understood by a comparison of Figures 10 and 11.

Figure 11 is a median optical section of a *Urosalpinx* embryo, after the ectoderm *F* has surrounded the food yolk *B* and the oral half of the latter has been absorbed. The oral end is uppermost, and the ventral surface to the left, and it is therefore in the same position as Figures 1, 4, 6 and 8. The layer of ectoderm has passed around the aboral surface of the food yolk and up its dorsal surface as far as the point *C* near the centre of the dorsal surface. A comparison with the previous figures shows that the point *C*, which in Figure 11 is the only portion of the body which is not covered with ectoderm, is the same as the point in Figure 1 where the dorsal surfaces of the segmentation spheres join the food yolk. A view of the dorsal surface of the same embryo, Figure 10, shows that the area *C* is circular and that its margin is formed by a ring of ectoderm cells, and deeper focusing shows that the oral margin and sides of this ring are in contact with the endoderm spherules *D* and its aboral margin in contact with the upper edge of the food yolk *B*. It thus forms a circular aperture, the gastrula mouth, through which the digestive cavity opens externally; it is functionally a mouth, and food passes through it into the digestive cavity before the formation of the definitive mouth. At this stage, Figure 11, the ectoderm of the oral end of the body has become differentiated into the foot *G*, the head vesicle *K* and the invagination *I*, which is to become the definitive mouth.

This invagination does not at first reach the surface of the endoderm, and its inner end is closed. Since it makes its appearance above the foot *G*, we are able to say that it is formed at the point which is indicated by the letter *A* in Figure 6, or at one end of the long axis of the embryo. Since the food yolk is not entirely covered by the blastoderm at this stage, (Figure 6,) it is plain that the mouth invagination is formed at that pole of the egg where segmentation commences, and which we have called the oral pole.



At the stage shown in Figures 10 and 11, the endoderm *E'* has grown upwards dorsally and at the sides, and the digestive cavity *E''* is nearly shut in, although there is still an uninclosed belt or zone along the ventral median line, represented by dotted lines in Figure 11. The greater part of the wall of the digestive cavity is so filled with small vacuoles that the outlines of the constituent cells could not be traced; but along the dorsal and lateral edges, where it meets the food yolk, the large endoderm spherules *D* can still be seen. In the dorsal view of the same embryo, Figure 10, the primitive urinary organs *H* are seen at the sides of the body, covered with a single layer of small nucleated polygonal cells and filled with a loose mass of nearly spherical cells, the origin of which was not traced.

At a later stage of development, the endoderm meets along the ventral median line, and the oral end of the embryo is then composed of two nearly concentric layers of cells, the ectoderm and endoderm, separated by a body cavity, which appears to be identical with the cavity of segmentation. For some time the floor of the digestive cavity is the surface of the food yolk *B*, but in time the endoderm grows inwards, as shown by the dotted lines at *E'* in Figures 10, 11 and 12, and separates the food yolk from the cavity. Before this floor is completed, the albumen of the egg capsule is drawn into the digestive cavity, through the gastrula mouth, apparently by ciliary action. The cavity becomes greatly distended and the long axis of the embryo lengthened, as shown in Figure 12.

The ectoderm is stretched by this inflation, and the foot *G*, Figure 12, and the mouth invagination *I* are thus rendered less conspicuous than at an earlier stage. The endoderm, on the contrary, absorbs nutriment from the food, thickens, and becomes filled with great numbers of oil-like vacuoles, and is more conspicuous than at an earlier stage.

Along the dorsal portion these vacuoles unite and form one large one for each cell, and the outlines of the separate cells become visible. The velum *L* makes its appearance as a band of large nucleated cells, with long cilia, and running across the anterior end of the body, dorsal to the mouth invagination. At *H* one of the primitive kidneys is shown, and it will be seen that it is much nearer the anterior end of the body than in Figure 10. At this stage the floor *E'* of the digestive tract is not quite completed, and near the dorsal surface a small portion of the food yolk is still exposed. The margins of

the gastrula mouth are now greatly thickened, and before the floor of the digestive tract is quite completed, the opening is obliterated and its margins become the shell gland, Figure 12, *C*, upon which a small circular transparent shell, *M*, soon appears. The closure of the gastrula mouth and formation of the shell take place before the invagination *I* of the true mouth unites with the endoderm.

Figure 13 is a somewhat older embryo, less highly magnified than Figure 12, but in nearly the same position. The marked change in the general form and outline of the body is the result of a ventral flexure of the long axis. The dorsal surface is thus lengthened, the ventral shortened, and the oral and aboral poles brought nearer each other.

The ectoderm is now continuous over the spot *C*, where the gastrula mouth was situated at an earlier stage, and the shell, which has now increased greatly in size, has moved from its primitive position over the point *C* and is now a symmetrical circular cap, *M*, upon the convex dorsal surface of the bend in the body of the embryo. Around its edge is a thickened ridge, *R'*, the rudimentary mantle.

The food yolk *B* is of about the same size as in Figures 10, 11 and 12, but it is now entirely shut off from the digestive cavity *E''* and lies between the integument *F* and the endoderm *E'*.

The mouth invagination now communicates by a short ciliated cesophagus, *I*, with the digestive tract, and the embryo draws into its stomach the yolk of abortive eggs and fragments of other embryos, as well as the transparent albumen. As the solid particles are drawn through the cesophagus, they are pressed and twisted into long strings, *N*, which are frequently to be found in the digestive cavity. The foot *G* is now quite conspicuous, and the velum *L* begins to bend towards the dorsal surface. The embryo now grows very rapidly, and at the stage shown in Figure 14, is three or four times as large as that of Figure 13, and is represented in substantially the same position. Up to this time the embryo has been bilaterally symmetrical, but the symmetry is now departed from by the twisting of the aboral pole towards the right side. In a side view, as in Figure 14, the outline is roughly triangular; one angle being formed by the head vesicle *K*, another by the food yolk *B*, and the third by the bend in the dorsal surface which is covered by the shell *M*; the dorsal surface forms two of the sides of the triangle, and the third, more broken side is formed by the outlines of both the ventral surface and the oral end of the embryo. Owing to the

twist which is mentioned above, the front view of the embryo is no longer symmetrical. At the earlier stage shown in Figure 13, a view from in front, or along the line *EB*, would be perfectly symmetrical, and the food yolk *B* would be hidden behind the oral end of the body. In a similar view of Figure 14, that is a view along the line *KB*, the whole of the food yolk *B* would be seen to the right of the head vesicle *K* and the velum *L*, as is shown in the somewhat older stage, Figure 15. In Figure 14 the shell *M* is still nearly symmetrical, and still rests like a cap upon the rounded angle formed by the flexure of the dorsal surface. The organs at the oral end of the body are now quite highly developed. The foot *G* is a large projection which contains a cavity, which is traversed in various directions by a network of contractile cells, with the central nucleated body and radiating processes so characteristic of the interstitial connective tissue corpuscles of the mollusca. Among them a few free white blood corpuscles may occasionally be seen; and as the foot changes its shape, through the contraction of the radiating processes, the blood corpuscles are driven from one point to another. The head vesicle *K* is similar to the foot in structure, but its cavity is larger, its contractions more regular, and its functions as an embryonic heart much more efficacious. No trace of a distinct layer of mesoderm, such as is readily recognized in this region of a fresh water pulmonate at this stage, could be detected. The velum *L* is now well developed, and its free ends nearly meet upon the dorsal surface of the neck. In a view from in front the halves of the velum are seen to project from the sides of the body, as in those Gasteropods where the veliger embryo leads an active life. The oesophagus is now a long cylindrical ciliated tube, slightly bent upon itself, so that the convex side of the bend is dorsal. The digestive tract is still a large unspecialized chamber, which fills nearly the whole embryo. During growth the endoderm cells continue to grow more conspicuous, and they also increase greatly in size, and are represented in Figure 14 about as large as in Figure 12, although the latter embryo is much smaller and more highly magnified.

The endoderm and ectoderm are in contact over the greater portion of the surface, except posteriorly, where they are separated by the food yolk *B*, and at the oral end of the body. At the point *O* a ciliated depression indicates the point where the anus is to be formed, and a thickening of the ectoderm, not shown in any of the figures, runs inwards to form the rectum. This is at first a solid

cord, and it becomes hollow and communicates with the digestive tract at a later stage. I was not able to determine how much of the intestine is formed from this plug of ectoderm.

By a comparison with the previous figures, the point *O* will be seen to be separated by the width of the food yolk from the point where the gastrula mouth was situated.

The subsequent changes of the now rapidly developing embryo are too complicated to be described without carefully finished drawings, but outlines are given of four of the most characteristic of my figures of the later stages.

Figure 15 is an embryo older and much larger and less highly magnified than Figure 14, and viewed from in front or along the line *KB*; in Figure 14 and Figure 16 a still older and larger embryo is shown from behind. The two folds of the velum, *LL*, now project considerably from the sides of the neck, and the relation of this organ to the head vesicle and foot will be best understood by a comparison of Figures 14, 15 and 16. In Figure 14 the head vesicle *K* is dorsal to the mouth, and in Figure 15 it is a nearly spherical chamber, *K*, in front of the velum *L*. In Figure 16 part of its outline, *K*, is seen in front of the velum. In Figure 15 the oesophagus *I'* and the buccal cavity *I* are seen through the head vesicle, and beyond them is the foot *G* and the outline of the neck. The primitive kidneys have now passed forward and project from the sides of the neck at *H*, in Figures 14, 15 and 16. In Figure 16 the foot *G* is a large creeping surface, anterior to which is the mouth opening *I*; at the point where the head vesicle joins the velum, the tentacles *V* have now made their appearance. In both figures the food yolk *D* is seen a little above the velum and upon the right side of the embryo. It is a little difficult to use the terms right and left in the description of an irregularly twisted form, but it will be seen that if either of these figures were rotated until the creeping surface of the foot were below, and the head vesicle anterior, the food yolk would be on the right side. In Figure 15 the shell is still nearly symmetrical, although it has increased greatly in size and now covers all of the body, except the portions which formed the two ends of the embryo in Figure 10. The endoderm cells have increased greatly in size, and are of the same apparent size as in the more magnified figures of earlier stages. Around the margin of the shell there is, as in earlier stages, a thickened rim, the mouth, and anterior to this, on the right side of the dorsal surface of the neck, the

integument becomes folded at *S* to form the mantle chamber. The edges of this chamber are slightly scalloped, and the projecting points are the beginnings of the gill filaments. Within this cavity a large rhythmically pulsating organ, *T*, the embryonic heart, now makes its appearance. The shell now becomes asymmetrical, the right side of the dorsal margin, that is the margin nearest the mantle cavity *S*, growing most rapidly; and the shell soon assumes a spiral form, as shown in Figure 16. A comparison of this with the preceding figure shows that the lip of the shell of the adult is the right margin and the columella the left margin of the embryonic cup-shaped shell. In the stage shown in Figure 16, the margin of the columella is bent outwards at *U*, thus forming a fold, which lies upon the posterior face of the body, or neck, just above the foot. This latter organ is at this stage placed at right angles to the long axis of the aperture of the shell, but it soon rotates so as to be parallel to this axis, as shown in Figure 18; at the same time it increases in size and becomes about as long as the shell. By this rotation and growth the upper surface of the foot is brought into contact with the columellar flap *U*, which grows and becomes the operculum. For some time this is united to the shell by an elastic hinge or line of flexure. When the foot is withdrawn into the shell, the operculum bends down with it into the aperture, bending along the line where it is reflected outwards from the columella, but it soon becomes separated from the shell, and the growth of the foot carries it away from the columella, as shown in Figure 18. The velum begins to become rudimentary at about the stage shown in Figure 16, and at the stage shown in Figure 17 it is quite small, and in Figure 18, the stage in which the animal escapes from the capsule, it has disappeared.

The large food yolk and the supply of food contained in the vase are sufficient to carry the young animal up to the true gasteropod form, and the free-swimming veliger stage of development is omitted, although the tendency to develop a velum is still retained.

The changes which we have described may now be briefly recapitulated, as follows:

Segmentation takes place at one pole, the oral pole, of the large yolk and results in the formation of a blastoderm.

Two kinds of segmentation spherules are distinguishable at a very early stage: large opaque spherules, which ultimately give rise to the wall of the greater part of the digestive tract, and much smaller transparent spherules, which soon become ciliated and form a layer

ventral to the endoderm and arching over the segmentation cavity. The endoderm spherules become arranged in a band around the sides and dorsal margin of the food yolk, and the layer of ectoderm extends over them and also down onto the ventral surface of the food yolk. The endoderm is also pushed down onto the sides of the food yolk with the growth of the ectoderm.

The foot now makes its appearance at the oral ventral end of the embryo.

The ectoderm surrounds the food yolk, and form the margins of the gastrula mouth, upon the dorsal surface.

While the digestive cavity still opens externally by the gastrula mouth, the true mouth invagination makes its appearance at that end of the embryo where the segmentation began. The digestive cavity is formed by the absorption of the oral half of the food yolk, and the walls of the cavity are derived from the macromeres. For some time the aboral floor of the digestive tract is formed by the surface of the food yolk, but the edges of the endodermal wall soon become reflected inwards and at last form a continuous floor which separates the food yolk from the digestive cavity. Before this floor is completed, or the true mouth communicates with the cavity, the margins of the gastrula mouth become thickened to form the shell gland, and the opening disappears. Soon afterwards the true mouth is formed, and later the anus and intestine, and a considerable portion of the latter is derived from the ectoderm.

At first the body is long, cylindrical and bilaterally symmetrical, and the mouth is at one end and the shell upon the dorsal surface, but it soon bends upon itself so as to shorten the ventral surface and bring the extremities nearer each other, and a second twist carries the aboral end of the body onto the right side, and the most posterior portion of the body is now the middle of the dorsal surface.

A velum is now developed, but soon lost, and the animal leaves the egg case as a true Gasteropod.

The developmental history here traced is of especial interest in its relation to the gastrula theory. The typical gastrula stage, resulting from total regular segmentation, as in the Echinoderms and Paludina or Cyclas, is an elongated double walled vase, with an opening at one end, and an axially elongated cavity.

The outer wall of transparent ectoderm is not differentiated into organs, and it unites with the endoderm, which is also undifferentiated, around the margins of the aperture.

It is clear that no stage in the present development is a typical gastrula stage, nor is there any stage which can be regarded as a specialized gastrula stage complicated by the formation of other organs; but a little study will show that the embryo presents at different periods all the phases in the formation of a gastrula, although there is no time when all the characteristics of a gastrula exist together. The gastrula *stage* has disappeared, but the gastrula *form* persists, and may be recognized by neglecting all those complications of structure which do not take part in its formation. Suppose, for instance, that the differentiation of the ectoderm of the oral end of the body, which in Figure 10 indicate the positions of the foot, mouth, velum and head vesicles, did not make their appearance until a later period, we should then have at this stage an undifferentiated layer of ectoderm, entirely surrounding the embryo, except at the point *C*. At the same time, imagine the development of the wall of the digestive tract accelerated, and the cavity entirely separated from the food yolk, before the specialization of the gastrula mouth as a shell gland.

We should then have a central digestive cavity, surrounded by two layers of cells, widely separated on one side by the food yolk.

If this were away, the embryo would then be a typical radically symmetrical gastrula. If the food yolk were wanting, the development of the wall of the digestive tract accelerated, the development of the foot, mouth invagination, velum and head vesicle retarded, we should have a true gastrula; or, conversely, the acceleration of the development of the latter organs, and the retardation of that of the digestive cavity, and the presence of a food yolk, might so modify a typical gastrula, as to give us the form of development which we here find.

## DESCRIPTION OF THE PLATE.

All the figures are from the embryos of *Urosalpinx cinereus*, excepting Figs. 6, 7, 8 and 9, which represent an early stage in the development of *Astyris lunata*.

The letters of reference are the same for all the figures, and are as follows :

- A. The *Germinative* or *Oral Pole* of the embryo.
- B. The unsegmented *Food Yolk* at the *Nutritive Pole* of the embryo.
- C. The point upon the dorsal surface of the embryo, where the *gastrula mouth* is situated during the stage of development shown in Fig. 11. At the stage shown in Fig. 12, the *shell-gland* occupies the area where the *gastrula mouth* was situated a little earlier.
- D. The large opaque segmentation-spheres, which form the *Endoderm*.
- D'. In Fig. 1, the more opaque dorsal endodermal portion.
- D''. In Fig. 1, the transparent ventral ectodermal portion.
- E. The endodermal wall of the digestive cavity.
- E'. The portion of this wall which is reflected onto the oral surface of the food yolk.
- E''. The cavity of the embryonic stomach.
- F. The *Ectoderm*.
- F'. That margin of the layer of ectoderm which grows down onto and around the food yolk.
- F''. The *Segmentative Cavity*.
- G. The *Foot*.
- H. The *Primitive Renal Organ*.
- I. The *Definitive Mouth*.
- K. The *Head Vesicle*.
- L. The *Velum*.
- M. The *Shell*.
- N. A mass of food which has been drawn through the mouth into the digestive cavity.
- O. The *Anus*.
- R'. The *Mouth*.
- S. The mantle cavity.
- T. The embryonic heart.
- U. The operculum.
- V. The tentacles.



**FIGURE 1.**—An egg, at a very early stage of segmentation, seen from the left side and magnified 100 diameters.

The egg is considerably lengthened along the axis which passes through the area of segmentation. Two segmentation spherules are sharply defined and project from the dorsal side of the oral end of the food yolk. Each of these is divided into a dorsal opaque granular portion, which is to give rise to the spherules of the endoderm, and a more transparent ventral portion from which the ectoderm is to be, in part, derived. A third segmentation spherule has become fused with the food yolk, ventrally to the two projecting spherules, and is destined to give rise to the greater part of the ectoderm.

**FIGURE 2.**—The germinative pole of an egg, at a later stage of development, viewed from the ventral side and magnified 250 diameters.

The food yolk, only a small part of which is shown in the figure, has regained its nearly spherical shape, and on its surface, just below the ventral edge of the layer of ectoderm, is seen the sharply defined transparent area which gives rise to new ectoderm spherules. Behind the layer of ectoderm *F*, the upper edges of the larger endoderm spherules are seen, and the smaller endoderm spherules *D*, *D*, extend around onto the sides of the area of ectoderm.

**FIGURE 3.**—The same egg, viewed from the dorsal surface.

The layer of ciliated ectoderm is now seen at *F*, projecting beyond the endoderm spherules *D*, *D*.

**FIGURE 4.**—Optical section of the same egg. Its dorsal side is towards the right.

By the addition of new spherules, derived from the food yolk, at *F'*, the ventral edge of the layer of ectoderm gradually extends down onto the food yolk, while its dorsal margin extends towards *C*, over the endoderm *D*, by the addition of spherules derived from the latter.

**FIGURE 9.**—This figure should come next in order, but, by an oversight, it has been incorrectly numbered. It is a ventral view of the oral pole of an egg at a later stage than Figure 2.

**FIGURE 9.—Continued.**

The layer of ectoderm *F* has spread over the endoderm spherules *D*, and begins to push them down onto the sides of the foot yolk.

**FIGURE 5.—The embryo of *Astyris lunata*, viewed from the dorsal surface, at a later stage than Figure 9.**

The layer of ectoderm has spread over the food yolk *B*, so as to nearly cover it. It has also covered the endoderm spherules *D* and pushed them down onto the sides of the food yolk.

**FIGURE 6. The same embryo, seen from the left side.****FIGURE 7. Optical section of Figure 5.****FIGURE 8. Optical section of Figure 6.****FIGURE 10. Dorsal view of a still older embryo of *Urosalpinx*.**

The ectoderm now entirely surrounds the foot yolk, except at the point *O*; the gastrula mouth. The primitive kidneys *H* project from the sides of the body.

**FIGURE 11 —Longitudinal section of the same embryo, in the same position as Figures 4, 6 and 8.**

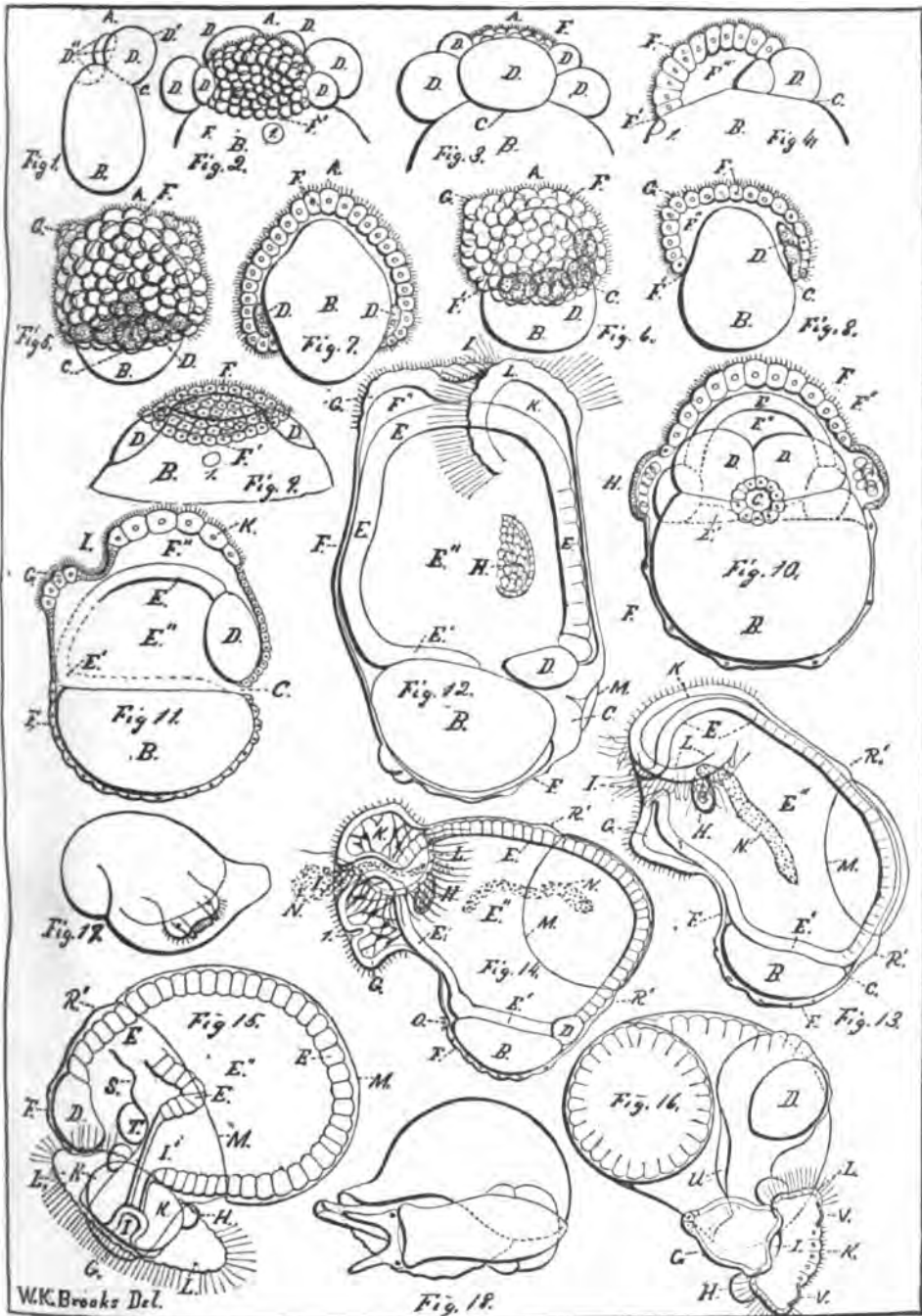
The head vesicle *K*, the mouth invagination *I*, and the foot *G*, have now appeared at the end opposite food yolk *B*.

**FIGURE 12.—An older embryo in the same position, showing, in addition to other features, the velum *L*, the shell gland *C*, and the shell *M*.****FIGURE 13.—Similar view of an older embryo, magnified only 150 diameters.**

The gastrula mouth has disappeared, the true mouth *I* has been formed, and the digestive cavity *E''* contains strings of food *N* which have been drawn into it through the short oesophagus.

**FIGURE 14.—A nearly similar view of a still older embryo, magnified 100 diameters.****FIGURE 15.—The anterior aspect of an older embryo.****FIGURE 16.—Still older embryo, viewed from below, magnified 75 diameters.****FIGURE 17.—The young gasteropod retracted within its shell.****FIGURE 18.—An older one, expanded, and seen from below. This represents the stage at which the young usually leave the capsule.**

# Development of Gasteropods.





JOHNS HOPKINS UNIVERSITY,

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STUDIES

FROM THE

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**ON THE RESPIRATORY FUNCTION OF THE INTERNAL INTERCOSTAL MUSCLES.** BY H. NEWELL MARTIN, *Professor of Biology in the Johns Hopkins University, Baltimore, U. S. A.,* and EDWARD MUSSEY HARTWELL, M.A. Plate I.

AN inspection of the ordinary text-books of Physiology is sufficient to shew that the part played by the internal intercostal muscles, in the production of the respiratory movements of the mammal, is still a subject upon which there is no agreement among Physiologists. Thus in the text-books accessible to us we find these muscles, in some cases with more or less reserve, described as inspiratory in function (Dalton, Ludwig, Vierordt); or as both inspiratory and expiratory in different portions (Carpenter, Flint, Hermann, Mc Kendrick); or as expiratory only (Donders, Funke); while Foster says that their action must at present be left an open question. This divergence of opinion induced us to attempt to solve the problem by a method which, so far as we know, has not hitherto been employed.

To arrive at a decision as to the function of these muscles as rib elevators or rib depressors, from a simple mechanical study of their attachments seems impracticable; on account of the irregular shape of the ribs and the doubt which must exist as to whether the upper or the lower rib, to which one of these muscles is fixed, is to be regarded as its origin or insertion. Moreover, if experiments be made in which any or all of the other muscles be cut away, then direct observation of the movement of the ribs which follows when the internal intercostals contract is useless so far as settling their function goes; because we do not know that we have not removed some muscles which, in ordinary breathing, held fast either the upper or lower rib and so determined either the inspiratory (rib elevating) or the expiratory (rib depressing) function of the muscle. The observation of the result of direct electrical stimulation of these muscles is also not decisive: unless all the other muscles which work with them in breathing be also excited in proper order and degree; which is impossible. Unless this be done, however, we cannot by this means tell which rib is normally the fixed one, when one of these muscles contracts. It

seemed to us, however, that, by isolating an internal intercostal and then observing whether it contracted simultaneously or alternately with the diaphragm, its function could be settled; since, from the general co-ordination of muscular contractions in the respiratory movements, there can be no doubt that muscles excited from the respiratory centre and contracting during respiration simultaneously with the diaphragm, are inspiratory muscles; and that those contracting when it relaxes, are expiratory.

Dogs and cats were employed in our experiments. The animals having been etherized, tracheotomy was performed and the apparatus for artificial respiration connected with the wind-pipe. The abdomen was opened by an incision along the *linea alba* and a transverse incision, so as to expose the diaphragm from below. The skin and the serratus and pectoral and other muscles were then dissected away from one side of the chest so as to lay bare the external intercostal muscles from the fourth or fifth to the ninth or tenth ribs: except where they were covered at their dorsal portions by the muscles running alongside of the vertebral column. During this operation several small vessels commonly required tying, especially in the dog.

One intercostal space, say that between the eighth and ninth ribs, was then selected and the anterior part of the external intercostal muscle divided, near its attachment to the lower of the two ribs, for from an inch to an inch and a half at its sternal end. The internal intercostal, which was carefully avoided during the operation, then remained alone, with the pleura uniting the front parts of the two ribs. The eighth and ninth costal cartilages and the tissues between them were next divided, the chest opened and the artificial respiration apparatus set at work. The tissues in the seventh and ninth intercostal spaces were then completely divided nearly all the way back to the vertebral column.

Next, from the pleural side, a fine-bladed knife was inserted between the eighth intercostal nerve and the eighth rib near the vertebral column and an incision carried forwards, without cutting the nerve, until it reached the outer end of the region where the external intercostal muscle had been divided. In making this incision we have found it impossible to avoid cutting the intercostal vein of the space operated upon; the artery was sometimes divided also, but this did not seriously impair the result. An incision of similar extent was then made along the upper border of the ninth rib, and finally a bit of both ribs corresponding in extent and position to these incisions was

completely cut away by bone forceps. By this means we obtained from an inch to an inch and a half of the sternal ends of the eighth and ninth ribs, united only by the internal intercostal muscle and the pleura, and connected with the rest of the body by a band of tissues consisting of the intercostal nerve (and artery) and some muscle and pleura.

The remaining sternal piece of one of the ribs, sometimes the upper, sometimes the lower, was then fixed in a clamp and placed in such a position that the band of tissues above referred to hung quite lax; so that movements of the ribs or even of the whole trunk, unless unusually powerful, could not, through it, drag on the piece of muscle to be experimented upon. A string was then attached to the other rib and passed over a pulley to a lever which carried a weight and extended the muscle. This lever carried a pen which wrote on the paper of a Ludwig's Kymographion. A tambour was fixed beneath the diaphragm and connected with another. This latter tambour was provided with a lever which recorded the contractions of the diaphragm immediately under the lever connected with the intercostal muscle.

The artificial respiration was then stopped, and the animal was generally found apnoeic. The further course of events differ in the dog and cat. In the former the diaphragm, when the apnoea passed off, made a few contractions without any activity of the intercostal muscle: but this latter soon began to contract in regular alternation with the diaphragm and before the occurrence of expiratory convulsions; in fact with the commencement of dyspnoea. Having made a few contractions, varying in number from fifteen to five or six, it again ceased its activity, although the animal became more and more dyspnoeic as evinced by the contractions of the diaphragm. This cessation seems due to the exhaustion of the muscle occurring rapidly from exposure and the interference with its blood supply; since the contractions become successively feebler before disappearing; and if artificial respiration be resumed and the animal kept alive until the muscle has had a period of rest, then on again stopping the respiration the phenomena are repeated, but the muscle makes a smaller number of contractions. We have seen this restoration occur five times in the same dog. The Figure, Pl. I. to be read from right to left, shews the tracing of the fourth set of contractions obtained in one experiment with the sixth internal intercostal muscle of a dog; the ascents of the upper line indicate contractions of the intercostal muscle; and those of the lower line contractions of the diaphragm. It will be seen that they alternate perfectly.

quite red, and at the same time sweat appears. The question arises, Is this due to a direct action of the nerve upon the gland-cells? or is it dependent on circulatory changes, such as have been supposed to produce the sweating of the horse's face seen after section of the sympathetic; or finally, does the irritation of the nerve merely throw the unstriated muscular fibres about the glands into activity and press out the glandular contents? This last idea is not correct, because although the contraction of surrounding muscular fibres might undoubtedly squeeze out some sweat from the glands, yet when this was wiped away no secretion could from this cause reappear in a continuous manner when the stimulation was kept up; and experiment shews that the secretion may be excited for hours by prolonged stimulation of the nerve. As to the circulatory theory we know through the researches of the lamented Bernard that the blood-vessels play an important part in the secretory mechanism of the submaxillary gland; but we also know from the work of Ludwig and others that variations in the blood supply are even in that case by no means the only, or even the primary, factors in the production of the secretion. The following experiments confirm the results of Luchsinger and others that this is true also with respect to the sweat-glands. Thus if the leg of a cat be amputated and some minutes afterwards its sciatic nerve be irritated (Exp. 1), a secretion of sweat breaks out on the foot. It may be supposed that the gland here uses up the blood necessarily remaining in the capillaries; probably it does, but it is hardly to be believed that vaso-motor changes can wholly account for a secretion excited under these conditions. When the blood-supply is cut off by compression of the artery of the limb, it is still possible to excite a sweat secretion, even after muscular rigidity has commenced to appear (Exp. 2 and 3). This makes it still more improbable that the secretion following the stimulation of the nerve is due to circulatory changes in the capillaries of the glands. It must rather be inferred that nervous impulses act directly upon the protoplasm of the gland-cells, causing them to evolve their secretion; and largely apart from circulatory phenomena. The vaso-motor changes alone are also insufficient to cause secretion. In many cats the vessels of the foot are stimulated by the nerve, but no secretion follows. And in a cat (Exp. 17) the sciatic of which had been divided proximally, rubbing the foot caused redness but no sweating, being dependent upon inability of the gland-cells to be set into activity by the action of muscarin started them into activity.

Still, as might be expected, the secretion stands in a certain dependence upon the blood-supply. In the Experiments 2, 3 and 4 the sciatic was irritated, and the time which elapsed between the commencement of the stimulation and the appearance of sweat was noted. A ligature, including all the tissues but the nerve, was then tightened around the limb and the nerve again excited in exactly the same manner. During the first ten or fifteen minutes after tightening the ligature, the "latent time" of the secretion, as we may call it, was but little altered; but during the second period of fifteen minutes it was considerably increased, whether the compression was only moderate or the ligature was so tight as to allow the muscles to become rigid. If the ligature was now loosened the time elapsing between stimulation and secretion was again diminished. Whether in these cases the delays were due to loss of activity by the gland-cells or to diminished irritability of the nerve is not easy to decide. It is known that when the blood is shut off from the nerves in a warm-blooded animal the motor-fibres lose their irritability in from ten to twenty minutes. Now this is about the time when the sweat-production begins to slacken, but it must be remembered that the secretory nerves seem in general to retain their activity for a considerable time after the motor-fibres have lost their irritability, so that we might be less inclined to impute the delay to them. On the other hand, the fact that in the sheep the parotid gland continues to secrete for some fifteen minutes after death, without the stimulation of its nerves, seems to shew that secreting cells may retain their vitality unimpaired for a considerable time after the cessation of the blood-supply. It may also be supposed that the delay was due, not to a loss of irritability on the part of the gland-cells, but to a scarcity in the supply of material. But in either case it is obvious that the activity of the glands is to a certain extent dependent on circulatory conditions.

It having been established that the sweat following the irritation of a nerve is primarily due to a direct action of this nerve on the gland-cell, I shall proceed to state what changes in the cell's protoplasm are produced by changes of the activity of the nerve. In a cat the sciatic was cut and the animal kept until the fifth day, when the balls of the feet were excised, and placed in absolute alcohol; sections were made when the specimens were hard enough, stained with Beale's carmine solution, and mounted in glycerine. In another cat the sciatic was exposed and feebly irritated for about two and a-half hours, when the balls of the feet were treated in the manner just described. In the

glands from the limb with its nerve cut five days previously, the cells were noted and compared with those of the irritated gland. It was found that the irritated cells were smaller than the resting cells, more granular as regards their protoplasmic contents, and more highly tinged with the carmine solution, although left in it the same time as the resting cells. Whether, as Heidenhain thinks, the more highly tinged irritated cell is due to a peculiar chemical attribute of the albumin of these cells as regards carmine, is a point difficult to substantiate as fully as might be wished. It strikes me, it might be due to physical causes in the osmotic properties of the tissue to carmine, just as readily as to a peculiarity of this albumin. However be the fact explained, there is no doubt that irritated cells are more highly stained than those of the resting cell, although subjected to the same re-agents for the same time. Figs. 1 and 2 (Pl. II.) represent the gland-cells after section of the sciatic for five days. Figs. 3 and 4 represent the cells of the irritated gland, whilst Figs. 5 and 6 represent the cells immediately after an operation on a cat, *i.e.* without previous special rest or prolonged stimulation. All are transverse sections—the objective D and ocular No. 4 of Zeiss being employed.

In addition to the excito-secretory apparatus there is an inhibiting nervous mechanism connected with the sweat-glands. When the sciatic nerve of a cat is divided and pilocarpin administered to the animal, an active secretion of sweat soon breaks out. Upon irritation of the peripheral sciatic this secretion is checked, and this result is still more marked if the electrodes be applied directly to the balls of the feet. It may be suggested that this checking of the secretion depends upon vaso-motor changes; but I have already shewn that ligaturing the artery of the limb only affects the secretion after the lapse of some ten or fifteen minutes, while in this case the result follows almost at once on the application of the stimulus. It seems probable to me that the multipolar nerve-cells described by Coyne<sup>1</sup> about the sweat-glands form a part of the inhibitory apparatus. If so, they must be under the control of fibres running in the sciatic, since as above stated stimulation of that nerve, under certain circumstances, calls them into activity. But the sciatic receives fibres from the spinal cord in two ways; some directly through the roots which unite to form it, some indirectly through the sympathetic, and it remains to decide which course the inhibitory fibres take. Experiments of Vulpian<sup>2</sup>, which I have repeated

<sup>1</sup> *Comptes Rendus*, Tome LXXXVI. p. 1276.

<sup>2</sup> *Ibid.*

and confirmed, shew that they pass through the abdominal sympathetic, although that nerve also contains excito-secretory fibres which gain the ascendancy upon the application of powerful stimuli. This view receives confirmation from an experiment (Exp. 5) in which I opened the abdomen of a cat and lacerated the tissue about the left abdominal sympathetic, when the *left* foot became dry (whilst the others were moist) and remained so next day, even when the psychical irritation of tying the animal down had bathed the other feet in a profuse perspiration. I then gave this cat a dose of pilocarpin, when the sweat broke out profusely in all the extremities except the left, where it did not appear for a considerable time. In another cat I divided the left sympathetic, and next morning noticed that the left foot was moister to a small extent than the corresponding right extremity, and when a small dose of pilocarpin was given, then the left foot began to sweat profusely, whilst the right did not until considerably later. Here the exciting fibres coming through the lowest lumbar nerves and first sacral into the sciatic, shew an unwonted activity in consequence of the absence of the normal restraining influence proceeding through the sympathetic fibres. The inhibitory action when once set up continues for some time: when a cat's foot is sweating from pilocarpin, local irritation will considerably diminish the secretion for some minutes. This inhibitory mechanism no doubt plays a part in the production of the hot non-sweating skin of fever. For, as I shall shew further on, heat is one of the most powerful excitants of the sweat secretion; as indeed we daily experience in hot weather. Vulpian believes that the centre of the inhibitory fibres lies in the medulla oblongata; but I have found stimulation of that region to excite active secretion (Exp. 18). Stimulation of parts of the brain in front has also no restraining effect, so far as I have seen.

The path from the spinal cord to the sciatic nerve, of the excito-secretory fibres seems to be double. As already stated, powerful stimulation of the abdominal sympathetic (Exp. 6) causes sweating of the foot on that side. Moreover when the spinal cord of a cat is divided between the eleventh and twelfth dorsal vertebræ, irritation of the last three dorsal and first two lumbar anterior roots excites sweating in the corresponding posterior extremity (Exp. 8). Now these roots do not directly enter into the sciatic at all, so that these excito-secretory fibres must run through the sympathetic, into that nerve. On the other hand, if the last lumbar and first sacral roots, which help to form the sciatic trunk, be irritated, sweating of the foot is also produced (Exp. 9).

In the fore limb the excito-secretory fibres pass through the sympathetic. Irritation of the median nerve causes sweating of the medial half of the foot, and of the second and third toes, and the medial half of the fourth. When the ulnar nerve is irritated the other half of the foot sweats, the ulnar half of the fourth toe and the whole of the fifth (Exp. 10). The exciting fibres in this case must pass through the brachial plexus, but they reach it through the *ganglion stellatum*; since after excision of that ganglion on one side, asphyxia, which is a strong excitant of sweat, will leave the ball of the corresponding limb dry, while those of all the other feet are bathed in profuse perspiration (Exp. 11). The fibres reach the ganglion, at least in part, from the fourth dorsal spinal nerve, since irritation of its anterior root causes sweating of the corresponding anterior extremity (Exp. 12).

Having traced the secretory nerves into the spinal cord, the next question which presents itself is, In what parts of the cord or medulla do they arise or have their proximate centres? Luchsinger's<sup>1</sup> view that the centres, or at least some of them, lie in the cord, is confirmed by the following experiments. If the cord be divided between the sixth and seventh cervical vertebræ and the animal be left to recover for some hours (Exp. 13), and then asphyxia be produced, all the feet will sweat. That the accumulation of carbonic anhydride does not excite the secretion by a peripheral action is shewn by Experiment 14, in which one sciatic was divided previous to asphyxiation, and in which the corresponding foot remained dry, while the others sweated. The action is also not reflex, for it occurs after section of the posterior roots, so we must conclude that the sweating of asphyxia is due to a central action on the spinal cord. When the cord is divided between the eleventh and twelfth dorsal vertebræ, sweating on the posterior extremities still takes place on the production of asphyxia, so that their sweat-centres must lie in the cord behind that point. Heat is another excitant of sweat, which acts centrally. If the cord be divided between the tenth and eleventh dorsal vertebræ and one sciatic be also cut, then (Exp. 15, 16) upon placing the animal in a chamber warmed to 50° or 60° C. all the limbs sweat but that one with its nerve divided. That the action is not reflex is shewn by the fact that if, after section of the cord in the above region, the sensory roots attached to its posterior segment be divided, the hind feet will still sweat on heating.

<sup>1</sup> Pflüger's *Archiv*, xiv. (1877), p. 369.



Sweat secretion however can also be excited reflexly, as is shown by Experiment 16, in which stimulation of the central end of a divided sciatic caused sweating of the opposite foot.

The administration of certain drugs throws the sweat-glands into exaggerated activity. Either muscarin or pilocarpin given subcutaneously produces profuse sweating of all the feet; and this sweating still occurs on the posterior extremities when the sciatic nerves have been divided recently. The excessive perspiration in each case is stopped by atropin. Muscarin and pilocarpin however seem to act upon different parts of the peripheral apparatus. In an animal with its sciatic divided nine days previously and in which electrical stimulation of the peripheral end of the nerve caused no sweating, a dose of muscarin produced it (Exp. 17). This makes it probable that muscarin acts directly upon the gland-cells. Pilocarpin on the other hand causes no sweating in the foot of a limb the nerve of which has been divided seven days previously, and probably acts rather upon some part of the nervous apparatus of the glands than upon the secreting cells themselves.

When we compare the sweat and the salivary glands we find many points of similarity. Muscarin and pilocarpin excite both by a peripheral action, and in Experiment 15 I noticed that the salivary secretion was excited as well as the sweat, and as the heating acts centrally in the latter case it is probable that it excites the submaxillary by a central irritation.

If we compare the course of the sweat-fibres with those of the vaso-motor system, they will be found to pursue a somewhat similar course. Thus the vaso-motor and sweat-fibres pass from the lumbar part of the cord, and some from its dorsal part, through the abdominal sympathetic to the sciatic. But in the fore limb the vaso-motor fibres pass through the third, fourth, and fifth anterior dorsal roots, the sweat only by the fourth (Exp. 12), and run in the *ganglion stellatum* to the brachial plexus, and then to the median and ulnar nerves. Further, sweat-centres like the vaso-motor centres are situated throughout the cord and medulla oblongata, for when the latter is irritated an abundant secretion of sweat occurs in all the extremities (Exp. 18).

Appended are some of the experiments upon which the preceding statements are based.

EXP. 1. Cat, killed—posterior extremity amputated, sciatic nerve irritated, when sweat began to appear in the ball of the foot; this was repeated, with a similar result, several minutes after death.

## EXPERIMENT 2.

Time.	Distance between primary and secondary circuits of du Bois coil.	Compression of blood-vessels by ligature involving all the tissues except the nerve.	Time it takes for the sweat to appear—the irritation being kept up till it did.
P.M.			Seconds.
3.10	0		10
3.15	...		10
3.16	...	severe compression	
3.20	...		10
3.25	...		10
3.30	...		11
3.40	...	limb stiff	15
3.45	...		15
3.50	...		30
3.51	...	ligature loosened	
3.55	...		15
4.0	...		15
4.5	...		10

## EXPERIMENT 3.

4.0	2		15
4.5	...		15
4.6	...	moderate compression	
4.10	...		15
4.15	...		15
4.20	...		55
4.25	...		65
4.26	...	ligature loosed	
4.30	...		45
4.35	...		15

## EXPERIMENT 4.

3.11	5		7
3.16	...		5
3.20	...		7
3.23	...	moderate compression	
3.25	...		7
3.28	...		10
3.33	...		12
3.38	...		12
3.40	...	ligature loosed	
3.44	...		10
3.49	...		8
3.54	...		7

Exp. 5. Cat, etherized. Abdomen opened and the tissues torn up about the left abdominal sympathetic, the wound closed; it was then noticed that the extremities were bathed in sweat, except the left, which was dry. Next morning, the left foot was much dryer and paler than the right, when the animal was fastened on the holder.

11.20 A.M. Right sciatic divided, and a small dose of pilocarpin given subcutaneously.

11.27 A.M. Sweating and salivation profuse; but no sweat was as yet seen in the left posterior extremity. Irritation of right sciatic retards the sweating in that foot, but when the electrodes are locally applied to the foot, the retardation of sweat is much greater and remains so for a few minutes after removal of the electrodes. The left foot after a time began to sweat, but feebly compared with that of the opposite side.

Exp. 6. Cat, etherized. Left abdominal sympathetic placed on Ludwig's electrodes and the wound closed, the end of the electrodes projecting—feeble irritation of the left abdominal sympathetic caused no sweating, although a current of the same strength would make a sciatic give rise to sweat. Strong irritation to the sympathetic caused the foot to become paler for several seconds, then it became red and sweating.

Exp. 7. Cat. Left abdominal sympathetic divided on the previous day at the level of the fourth lumbar vertebra. The left foot became moister than the corresponding right one.

9.45 A.M. A small dose of pilocarpin was given.

9.52 A.M. The saliva is running, and the sweating in the left foot is more profuse than in the right, being generated more rapidly after being wiped off. The right foot sweating but little.

Exp. 8. Large cat, etherized. Cord divided between the eleventh and twelfth dorsal vertebra. Lumbar portion exposed. The nerves divided outside the dura mater, irritation of the anterior root of the tenth dorsal produced no sweating, whilst irritation of the three last dorsal and the first and second lumbar did—the lumbar nerves giving the most sweat. The third lumbar gave no sweat when irritated. Care was taken that there was no spreading of the electrical current.

Exp. 9. Cat, etherized. Cord divided in upper part of lumbar region, the peripheral ends of the two last lumbar, and first sacral nerves were irritated after having been divided outside the dura mater. The lumbar region of the cord was then excised. Stimulation of the last lumbar and first sacral nerves—anterior roots—caused sweating in the corresponding foot.

Exp. 10. Cat, etherized. Median nerve irritated, when sweat was seen on about half of the ball of the foot, median side, and on the second, third, and medial half of the fourth toe. When the ulnar nerve was irritated, sweating was seen in the ulnar half of the fourth toe, and the whole of the fifth.

Exp. 11. Cat, etherized. Artificial respiration kept up. The first two ribs resected after previous ligature of the venous trunks. The right stellate ganglion exposed, and a ligature tightened around it, when asphyxia was induced, but no sweating ensued in that anterior extremity, although present in all the others.

Exp. 12. Cat, etherized. Dorsal region of the vertebral canal opened, and the third, fourth and fifth dorsal nerves—their anterior roots—irritated, the fourth dorsal only producing sweat in the anterior extremity.

Exp. 13. Small cat. Cord divided between the sixth and seventh cervical vertebræ. After the lapse of a few hours asphyxia was induced, when all the extremities became covered with sweat.

Exp. 14. Cat, etherized. Sciatic divided, cord divided in the dorsal region—asphyxia induced, but no sweating was seen in the extremity with the cut sciatic, although it appeared on the other feet.

Exp. 15. Cat, etherized. Cord divided between tenth and eleventh dorsal vertebræ at 12.25 P.M. Right sciatic divided.

2.45 P.M. The animal was placed in a heated chamber.

2.55 P.M. Saliva flowing in a stream.

3.15 P.M. Temperature 50° C. All the feet sweating except the one with a divided sciatic.

Exp. 16. Cat. Cord cut in dorsal region on the previous day.

9 A.M. Placed in a warm chamber.

9.30 A.M. Thermometer 60° C. Sweating in all the extremities except the cut one. Animal removed.

9.40 A.M. Central end of sciatic stimulated, when sweat was seen to break out in the opposite posterior extremity.

Exp. 17. Cat. Nine days after section of sciatic the nerve was stimulated at its peripheral end, but no sweat appeared.

10.42 A.M. A drop of muscarin was given subcutaneously, when sweat appeared in the cut extremity.

Exp. 18. Cat, etherized. Medulla oblongata bared. Artificial respiration kept up. Medulla oblongata was irritated, when sweat broke out in all the extremities.

Exp. 19. Cat, etherized. Trephined and surface of cerebrum irritated at the centre for the retraction and abduction of the fore-paw, just behind the crucial sulcus in the superior external convolution, but no increase of the sweat secretion was noticed in the opposite anterior extremity.

Exp. 20. Cat. Cord divided between sixth and seventh cervical vertebræ, right sympathetic divided, and left sciatic. After a rest of some hours asphyxia was induced, when sweating appeared in all the extremities except the one with cut sciatic.

## II. Vaso-dilator Centres.

RECENT researches have led many physiologists to adopt the view that dilation of the blood-vessels is brought about by vaso-dilator nerves connected with vaso-dilator centres in the spinal cord. The object of the following experiments was to determine more accurately

the position of these vaso-dilator centres. Method.—The animals operated upon were cats with unpigmented feet. They were etherized, the cord bared by trephining and then divided, and then sometimes destroyed *in toto* for some distance; this was done by thrusting a wire between two trephined openings. The complete destruction of the cord was confirmed by *post-mortem* examination. The animals were usually operated upon in the morning, and heated in the evening; a few were kept till the next day and subjected to heat. The temperature was elevated in the chamber to over 50° C. unless the changes took place at a lower point.

I find that the centre of the vaso-dilator nerves for the skin of the posterior extremities is located between the 10th dorsal and the 1st lumbar vertebræ. To obtain this result the spinal cord was divided between the 6th and 7th cervical vertebræ, the right cervical sympathetic in the neck divided just above the first rib; then the left sciatic was cut and the animal allowed to rest till evening. Then it was placed in a chamber and heated, when the right posterior extremity became more red than the left posterior extremity. This experiment left no doubt that the skin of the posterior extremities was supplied with vaso-dilator fibres arising in the spinal cord. In another cat the right cervical sympathetic was cut just above the first rib, the cord divided between the 2nd and 3rd dorsal vertebræ, and the left sciatic divided, when upon heating the same colour-changes ensued as in the previous experiment. In another animal the cord was divided between the 6th and 7th dorsal vertebræ and the right cervical sympathetic cut just above the first rib; the left sciatic also divided. After a rest of several hours the animal was heated, and still the same colour-changes as above ensued in the posterior extremities. These experiments make it probable that the vaso-dilators for the feet arise from nerve-centres seated below the 6th dorsal vertebra. If so, then extirpation of the part of the cord containing them should abolish the vaso-dilator changes in the skin of the posterior extremity. In a cat the cord was destroyed between the 9th dorsal and the 1st lumbar vertebræ and the animal kept till next day. Upon heating no difference was noted in the colour of the feet, the one with cut sciatic and the other with it intact had the same colour. If anything, the foot with sciatic cut was always slightly more red than the one whose sciatic was not divided. In another animal the cord was destroyed in the morning between the 10th dorsal and the 1st lumbar vertebræ and the sciatic of one foot divided. Upon heating in the evening and next

morning no colour-changes took place except those due to heating in feet with sciatics divided, that is they become paler.

I also noticed rhythmical changes of colour about every thirty seconds in the foot with uncut sciatic, during heating. These changes also take place without heating. Now Lovén has seen in experimenting on rabbits that their saphenous artery dilates and contracts rhythmically about twice every minute. These rhythmic changes of colour in the ball of the foot would correspond to this number.

The inquiry arises here, How does heat excite the centre of the vaso-dilator fibres? It has been shown that heat can excite by a direct action on centres in the spinal cord, as is the case in regard to the sweat-centres. On the other hand Grützner<sup>1</sup> has proved that heating the central end of the sciatic throws the vaso-dilators into activity. It is probable that the heat here acts directly on the centres in the cord, and thus dilates the blood-vessels of the skin of the posterior extremities; but the action may also be reflex.

Appended is an account of some of the experiments.

Exp. 1. Cat; left sciatic divided, when the foot on that side for the first few minutes became paler than the opposite one; afterwards it became more red than its neighbour. The animal was then placed in a chamber heated to 33° C., when the cut foot became pale and the uncut extremity red.

Exp. 2. Cat; left sciatic divided, cut extremity more red than the opposite one. The animal was heated in a chamber, and immediately afterward the peripheral end of the divided sciatic electrically irritated, when the foot became pale. When the trunk of the uncut sciatic was electrically irritated, the foot remained red.

Exp. 3. Cat; right sciatic divided, section of spinal cord at the junction of the last dorsal and 1st lumbar vertebræ. Upon heating, the cut extremity became pale, the uncut red. Then the left abdominal sympathetic was divided (the abdomen being opened), and the animal again heated, when no difference in colour was found between the two feet.

Exp. 4. Cat; right cervical sympathetic cut just opposite a section of the spinal cord between the 6th and 7th cervical vertebræ; left sciatic divided. Animal left to rest for several hours. Upon heating the chamber to 43° C. the cut extremity became pale whilst the uncut became more red.

Exp. 5. Cat; cord divided between the 6th and 7th cervical vertebræ, right cervical sympathetic divided opposite the section of the cord, left sciatic divided and the animal heated to over 50° C., when the uncut foot became more red than its neighbour, whilst the cut extremity became paler than it was before. The ball of the uncut foot was also noted to rhythmically change colour, that is become pale and then red. These changes were accomplished in about every thirty seconds during the heating.

<sup>1</sup> Grützner. Pflüger's *Archiv*, Bd. xvii. 1878, p. 229.

Exp. 6. Cat; cord divided between 2nd and 3rd dorsal vertebræ, the right cervical sympathetic divided low in the neck, just above the first rib, left sciatic divided. Upon heating the cut extremity became paler, whilst the uncut became more red.

Exp. 7. Cat; cord destroyed on previous day between the 9th dorsal and 1st lumbar vertebræ, left sciatic divided. Next morning upon heating to over 50° C., the foot whose sciatic was divided was a little more red than the opposite one.

Exp. 8. Cat; cord destroyed on previous day between the 10th dorsal and the 1st lumbar, right sciatic divided. Next day the foot with cut sciatic more red than its neighbour. Upon heating to over 50° C., the foot with cut sciatic was still more red than the opposite one.

### III. Rhythmical Functions.

It was first noticed by Gluge<sup>1</sup> in a paralyzed rabbit, which had been accidentally wounded in the lumbar region, that rhythmical contractions and dilatations of the sphincter ani occurred. These movements were not accompanied with any discharge of intestinal contents. Goltz<sup>2</sup> afterwards, unaware of Gluge's observation, noticed in the dog after division of the cord at the junction of the lumbar and dorsal regions that similar movements took place, and he found that irritation of the sciatic arrested them. Their number in the dog was about twenty per minute. Wishing to see if the centre in the cord for these rhythmic movements was the same as that on which the normal tonic contractions of the sphincter depend, I employed the following method:—Cats and dogs were selected and etherized, the cord was then laid bare (by trephining a small hole in the bony vertebral canal), and divided. The animal was allowed to rest some hours after this before the observations were made.

When the cord is divided very soon are to be seen rhythmical movements of the sphincter ani, which in the cat number from twenty to twenty-five per minute. If the abdominal aorta is compressed these become more frequent, but finally the sphincter passes into a state of relaxation. If a probe be inserted into the rectum the rhythmical movements are temporarily increased, or if not present are immediately set up. If in a dog a "dropper" is inserted into the rectum and attached to a Marey's tambour the rhythmic movements can be

<sup>1</sup> Gluge. *Bull. de l'Acad. Royale de Belgique*. 1868.

<sup>2</sup> Goltz. *Pflüger's Archiv*, Bd. VII. 1873, p. 582.

recorded; the sphincter ani relaxes, and remains in this state till the stoppage of the irritation, and then immediately makes a strong contraction, followed by several rhythmic contractions. The contraction immediately after the irritation passes off is much stronger than the one just before the stimulation.

As tracings of these movements have not hitherto been published I give one in Fig. 1. The figure is to be read from right to left. It will

Fig. 1.



be seen that at the moment (a) the stimulation of the sciatic commenced the movements ceased, but commenced again more powerfully (b) when the irritation of the nerve was discontinued. When the movements have been absent for a time, as is often the case, they are at first more powerful and considerably less frequent than they afterwards become. They gradually diminish in force and increase in frequency until they again cease; to recommence after a time either spontaneously or upon the application of a stimulus to the anus.

Moreover, I have found that the rhythmic contractions are not confined to the anal sphincter, but are also to be seen in that of the vagina of the cat and bitch. The number of these rhythms in the cat's vagina in one experiment was about four per minute, and independent of those made by the anal sphincter of the animal at intervening periods. For the vaginal sphincter has movements simultaneous with those made by sphincter ani and probably dependent upon traction from it. If the vaginal mucous membrane is irritated the rhythmical movements are increased in number. They are slowly produced and the relaxation occupies about the same time as the contraction of the muscle. When the central end of the sciatic is irritated then the rhythmical movements of the sphincter vaginæ cease.

As to the seat of the ano-spinal centre Masius<sup>1</sup> found that in dogs the spinal cord contained it at the union of the middle and inferior

<sup>1</sup> Masius. *Bull. de l'Acad. Roy. de Belgique.* 1867, 1868.



thirds of the 5th lumbar vertebra. When a section was made here he obtained a relaxation of the sphincter ani and reflex contractions ceased. In rabbits he found it at the level of the intervertebral disc between the 6th and 7th lumbar vertebrae. He also discovered that the sphincter ani was called into activity by fibres coming through the second and third sacral nerves. Irritation of the spinal cord, medulla oblongata, cerebral peduncles and thalami optici caused the sphincter ani to contract strongly in the rabbit.

In the cat I find all vaginal and anal rhythmic and reflex movements cease when the cord is divided between the 6th and 7th lumbar vertebrae. I divided the cord farther forwards on the previous day so as to allow the reflex functions to become more marked, and then found that section between the 5th and 6th lumbar vertebrae had no effect, while section between the 8th and 7th abolished the movements. I have not been able to divide the cord in such a manner that the tonic contraction should continue without the rhythmic doing so. The centres must be either one and the same, or if different located near each other. The vagino-spinal centre is also located between the 6th and 7th lumbar vertebrae, for when the cord is divided here all reflex activity in the sphincter vaginae is lost. At no time have I been able to separate the ano-spinal centre from the vagino-spinal. So these centres must be seated very near each other. That the rhythmic contractions are not due either to the inherent property of the muscular fibres of the sphincters or to the action of hypothetical ganglia located in them, is proved by experiments where the ano-spinal centre was destroyed and the animals lived some days. In these cases no rhythmic or other movement was seen or could be excited, save that due to the elasticity of the muscle itself. This leaves no doubt but that the spinal cord can originate impulses which cause rhythmic contractions of muscular tissue. The movements in question are not due to any action through the abdominal sympathetics, for these may be cut without interfering with them. When the vaginal or rectal mucous membranes are irritated I have seen the posterior extremities of one side execute movements of adduction and extension rhythmic in their nature.

The inquiry arises here, Do these movements of rhythmic dilatation and contraction normally take place either in lower animals or in man? When the act of defaecation takes place in the horse, it is seen that after the act the sphincter makes a series of rhythmic movements due to the excitation of the rectal mucous membrane having set the rhythmic apparatus into activity, but soon the over-

powering inhibition from the brain stops the rhythmic movement. Further, in man, when afflicted with dysentery and tenesmus, the sphincter will at the time of the tenesmus set up a series of rhythmic movements. In fact, I think it may be safely assumed that any strong excitation of the rectal or vaginal mucous membrane will cause their sphincters to exert themselves in a rhythmic manner. Thus we know that a suppository of opium is the best remedy for tenesmus, benumbing the sensory nerves of the part which convey the impulses from the locally inflamed portion.

The fact that the sphincter vaginæ is under the power of a reflex centre in the spinal cord is of some import in pathology. In the beginning of a prolapsus uteri the pressure of the womb will call the vagino-spinal centre into activity, and retard the downward inclination of the womb. It also offers an explanation of the prolapse so often seen in women with ruptured perineum, because the sphincter vaginæ being broken no support by reflex-contraction can be given to the womb in its retrograde changes immediately after child-birth. It also explains those cases of vaginismus due to a local hyperæsthetic state of the vaginal mucous membrane calling the vagino-spinal centre into unwonted activity. The fact of the spinal cord originating rhythmic movements has several suggestive bearings on the vaso-motor rhythm, and possibly other rhythmical movements.

The observation that irritation of the vaginal or rectal mucous membrane may call out in the voluntary muscles a rhythmic adduction and extension, is also of value in pathology in cases where we find rhythmic movements of an extremity taking place such as have been so thoroughly studied by Dr S. W. Mitchell. These cases can be explained, at least some of them, by the inhibition of the brain on the spinal centres being removed by disease, when the spinal centres take on a rhythmic activity; or, there may be a strong visceral irritation calling out rhythmic movements of an extremity by a reflex action on the centres of the spinal cord, or the spinal cord itself may contain an irritative lesion.

Appended are some of the experiments upon which the preceding observations are based.

Exp. 1. Cat; cord divided between the 1st and 2nd lumbar vertebræ, both abdominal sympathetics had been divided two days previously by opening the abdominal cavity; rhythmical contractions of the sphincter ani were seen to take place, irritation of rectum with a probe increased them, irritation of vaginal mucous membrane also increased the rhythm of the sphincter ani.

The sphincter vaginae was also seen to make independent rhythmical contractions about four per minute; it also made others simultaneous with those of the sphincter ani; irritation of the rectum with a probe caused the sphincter vaginae to open. Sciatic irritation by the induced current of the central end of the nerve arrests the rhythm of both sphincters.

Exp. 2. Cat; spinal cord previously divided, compression of aorta increased the rhythmic movements of sphincter ani and then paralyzed it. When section of the cord was made between the 4th and 5th, and 5th and 6th lumbar vertebrae, the rhythmical movements still continued as regards the sphincters. When, however, the section was made between the 6th and 7th lumbar vertebrae all rhythm of the sphincters ceased, nor could reflex movements be excited in them.

Exp. 3. Dog; cord divided on previous day in the lower end of the dorsal region, rhythmical movements of the sphincter ani numbered about 20 to 25 per minute, irritation of rectum by a probe increased them. Irritation of central end of sciatic arrested the rhythm at once, and when irritation caused the sphincter ani immediately contracted considerably, commencing a series of strong rhythmical contractions.

Exp. 4. Cat; cord divided at lower end of dorsal region in the morning, in the afternoon rhythmical movements of the sphincter ani and sphincter vaginae nearly simultaneous, beginning with the sphincter ani. The sphincter vaginae however had movements independent of the sphincter ani: mechanical irritation with a probe of either the rectum or vagina caused a rhythmic flexion and extension of one posterior limb usually, although sometimes both participated.

Exp. 5. Cat; cord previously divided in the dorsal region; cord divided at the 6th lumbar vertebra, when all rhythmic and reflex movements ceased in both anal and vaginal sphincters. A wire was thrust into the cord at each section so as to destroy it just above the point of section.

Exp. 6. Cat; cord divided between 3rd and 4th lumbar vertebrae, when the rhythmical movements of the sphincter ani could be seen. Section was made between the 5th and 6th lumbar vertebrae, and still the movements continued. When, after a rest of a few hours, section was made between the 6th and 7th lumbar vertebrae all reflex and rhythmical movements of the sphincters ceased.

Exp. 7. Cat; cord divided between 5th and 6th lumbar vertebrae in the morning, in the evening the rhythmic movements of the sphincter ani and sphincter vaginae were quite active. The cord was bared by a small trephine, and divided between the 6th and 7th lumbar vertebrae, when both the sphincters were paralyzed.

Exp. 8. Cat; cannula bound in ureter and connected with pressure-bottle to fill the bladder; section of spinal cord between 4th and 5th lumbar vertebrae. When pressure by a column of water was made on the mucous membrane of the bladder, the sphincter vaginae was set into rhythmical movement.

Exp. 9. Cat; spinal cord divided between the 5th and 6th lumbar vertebrae, when a wire was thrust down destroying the ano-spinal centre. Next day the sphincter ani had gradually drawn itself loosely together, but had no

tone whatever. Neither tonus nor rhythmical movements could be excited up to the death of the animal on the third day.

Exp. 10. Small cat; spinal cord divided between the 5th and 6th lumbar vertebræ, and then ano-spinal centre destroyed. During the course of 24 hours the sphincter ani drew itself slowly and loosely together. The animal was preserved for nine days, but neither rhythmic movements nor tonus appeared.

#### IV. Genito-Urinary Functions.

Whether a sphincter vesicæ truly and specifically exists has been considerably debated and studied. When I use the word "sphincter vesicæ" I refer to a muscular mechanism assisting to close the bladder whether a true sphincter or not. That the nervous system has an influence over a sphincter vesicæ in this sense has been known for considerable time and experimented on by several observers.

Budge<sup>1</sup> studied the nerves which throw the bladder and annexes into activity. In dogs, according to him, there is a genito-spinal centre about the 4th lumbar vertebra, which presides over the bladder and sexual organs. This lower genito-spinal centre is seated at a similar place in rabbits. He also has discovered a higher genito-spinal centre located in the pedunculus cerebri.

Giannuzzi<sup>2</sup> repeated the experiments on the centre for the bladder, and found at the level of the 3rd and 5th lumbar vertebræ, that contraction of the bladder could be called out by irritating the spinal cord.

Masius<sup>3</sup> of Liege found that by dividing the cord between the 1st and 2nd, 2nd and 3rd, 4th and 5th, and between 5th and 6th lumbar vertebræ that the rabbit retained his urine; but when the whole segment of the cord below these points was destroyed the urine escaped in a continuous manner. When the cord was cut in the rabbit between the 6th and 7th lumbar vertebræ the sphincter vesicæ was relaxed, although on the next day the bladder was filled and distended. In many rabbits he destroyed the cord at the inferior third of the 2nd lumbar vertebra, when the urine escaped, although the bladder was never entirely empty, this retention being due to the pressure on the urethra of fæcal matters accumulating in the paralyzed rectum; when these were removed the urine escaped. If in dogs the cord was cut at

<sup>1</sup> Budge. *Physiologie*.

<sup>2</sup> Giannuzzi. *Journ. de la Physiol.* 1863.

<sup>3</sup> Bull. de l'Acad. Royale de Belgique. 1868.

the 5th lumbar vertebra the bladder became distended; but when section was made below the point about the posterior border of the 5th lumbar vertebra the urine flowed, although the bladder retained a considerable quantity. He also submitted the sphincter vesicæ to pressure of water through the ureter, and, as he thinks, found that the force of contraction of the urethra did not diminish in a very sensible manner till he destroyed in the rabbit the cord at the inferior part of the 7th lumbar vertebra, and in dogs at the level of the hinder border of the 5th lumbar vertebra.

Masius states that the genito-spinal centre of Budge presides over the contractions of the bladder, and is not to be confounded with his centre, which governs the tonic and reflex contractions of the bladder's sphincter and is seated below the ano-spinal centre.

Kupressow<sup>1</sup> has also located the centre for the bladder in rabbits between the 5th and 6th lumbar vertebrae. He used pressure of water through the ureter to overcome the contraction of the bladder's sphincter.

Budge explains these results by the fact of the nerves of the bladder running up and ending in the cord at the 4th and 5th lumbar vertebrae, so that when a section is made at the lower end of the 5th lumbar vertebra the nerves of the bladder are cut. Budge also holds that the sphincter vesicæ in a strict sense does not exist.

My experiments on this subject were made in three ways. First the cord was divided on the previous day, and then the vertebral column was trephined and sections made at various levels, and the point noted where the urine began to flow, always cutting from above downwards. The second method was to expose the bladder, bind a cannula into it at the ureter's opening, and allow a current of warm water to run into it, and note the pressure, on a mercurial manometer attached by a side-tube, at which the water began to drop from the urethra. Then the cord was divided at different heights going from above downward, and the pressure (which had meanwhile been taken off by clamping the tube and lowering the pressure-bottle) was turned on, and that necessary to overcome the sphincter again noted. The third plan was to bind the cannula into the urethra, instead of into the ureter, and note the pressure under which the water forced its way into the bladder. The animal was etherized in all the experiments. Before running the water into the bladder the abdominal muscles were divided by a crucial incision so as to prevent any action on the bladder. I found no difficulty from the action of the detrusor.

<sup>1</sup> Kupressow. *Pflüger's Arch.* Bd. v. 1872, p. 291.

By an examination of the experiments appended it is seen that the genito-spinal or rather the vesico-spinal centre is seated in the cat and rabbit between the 5th and 6th lumbar vertebræ. This result is in opposition to the results of Masius. The experiments of Budge and Giannuzzi simply determined the nerves which, when irritated, call the detrusor into activity and thus overcome the sphincter. The experiments of Kupressow are in full accord with those made by me.

The inquiry arises here how to explain the experiments of Masius. In my experiments where I destroyed the spinal cord below and above the 7th lumbar vertebra for some distance, I saw, as Masius did, that part of the urine was retained, by reason of the elasticity of the sphincter itself. When he destroyed the cord above this point, and saw the urine flow upon the destruction of his vesico-spinal centre, then he assumed he had destroyed the centre. But has not the urine been previously retained by the simple elasticity of the sphincter? It is known that nerves coming through the sacral call the bladder into activity when irritated, which fact I have several times verified; and probably Masius, after destruction of the cord above when no urine flowed, caused its subsequent flow by calling these fibres into activity, and thus emptied the bladder. This I believe to be the correct conclusion to be drawn from his facts, and not that the genito-spinal centre is seated there. In an experiment of Masius on a dog, the pressure required to overcome the sphincter after section between the 5th and 6th lumbar vertebræ fell from 65 centimetres (water) to 20 centimetres, and after death was only 20 centimetres. His pressure-experiments on rabbits are not so conclusive as those of Kupressow nor so many. They are not so conclusive because the section of the cord appears to have reduced the necessary pressure greatly by shock, and this pressure too nearly approximates to that due to the elasticity of the sphincter itself to draw inferences.

The following protocols give the details of some of my experiments.

Exp. 1. Small cat; spinal cord divided in dorsal region two days before. The pressure is in millimetres of mercury.

Before section pressure necessary to force water through urethra from the bladder .....	140 mm.
Section between 4th and 5th lumbar .....	120 "
" " 5th and 6th " .....	30 "
" " 6th and 7th " .....	20 "
After death .....	10 "

Exp. 2. Very strong male rabbit.

Before section of cord .....	50 mm.
Section between 5th and 6th lumbar vertebræ .....	30 "
After death .....	10 "

Exp. 3. Cat; cord divided farther forward on previous day; when section of cord was made between 4th and 5th lumbar vertebræ no flow of urine followed. When, however, the section was made between the 5th and 6th lumbar vertebræ the urine began to flow.

Exp. 4. Cat; cord divided in dorsal region on previous day; when cord was divided at the 6th lumbar vertebra the urine flowed, no flow on previous section above that point.

Exp. 5. Male rabbit; cannula bound in urethra, and pressure made till the bladder began to fill with warm water.

Before section .....	36 mm.
Section between 3rd and 4th lumbar vertebræ .....	36 "
Section between 5th and 6th " " .....	30 "
Section at 6th .....	20 "
After death .....	16 "

Exp. 6. Male rabbit; same method as in preceding experiment.

Before section of cord .....	30 mm.
Section between 3rd and 4th lumbar .....	30 "
Section between 5th and 6th " " .....	30 "
Section at 6th .....	20 "
After death .....	16 "

Exp. 7. Small cat; cannula in the ureter.

Before section of cord pressure necessary .....	40 mm.
After section of cord between 3rd and 4th lumbar .....	30 "
After section of cord between 5th and 6th lumbar .....	20 "
After death .....	10 "

Exp. 8. Cat; cord bared by trephine, cannula bound into the ureter.

Pressure before section .....	40 mm.
After section between 4th and 5th lumbar .....	40 "
After section between 5th and 6th lumbar .....	24 "

Exp. 9. Cat; cord divided between 6th and 7th lumbar, and wire thrust down so as to destroy the cord below; the bladder was filled next day.

Exp. 10. Cat; cord divided between 6th and 7th lumbar vertebræ, and wire thrust down so as to destroy the cord below; next day the bladder was full of urine.

## V. Path of Secretory and Inhibitory Fibres.

The experiments of Dittmar, Mischer, Nawrocki, and Woroschiloff have demonstrated that in rabbits the motor, sensory, and vaso-motor tracts run in the lateral columns. The course of the vaso-motor path can be proved by taking cats with unpigmented feet and then making a section of the lateral columns, when the posterior extremities will become more red than the anterior. In another section I have shown that the spinal cord contains nerve-centres which preside over the secretion of sweat. Now these centres are connected together by fibres running down the cord, just as the motor-centres are linked together. To find out the path of these commissural fibres, it is necessary to make incomplete sections of the cord. Method: Cats with unpigmented feet were selected, etherised and the various columns of the cord divided in the dorsal region usually between the 6th and 7th dorsal vertebræ. I divided the cord here because it has been shown by my previous researches that sweat-fibres run through the three last dorsal nerves into the abdominal sympathetics. Hence any section below this point would not include all these fibres. The bony vertebral canal was opened by a trephine and cutting forceps, the bleeding being checked by styptic cotton and sometimes artificial respiration. Then the instrument of Woroschiloff was fastened on the bony vertebræ, and the cutting knives placed by the aid of a magnifying glass over the columns to be divided. The spinal dura mater was next carefully divided, the knife driven through the cord and the section then made. After the section the instrument was removed, and the wound immediately sewed up. The animal was placed in a warm place and left to recover from the anæsthetic. At the end of five hours it was bound down, the medulla oblongata was divided just below the point of the calamus scriptorius, and irritated by an induction-current derived from a du Bois apparatus run by a single Daniell cell. Artificial respiration was kept up before the section of the medulla to diminish bleeding and afterwards to preserve the life of the animal. The effects of the irritation of the medulla were noted. At the close of the experiment, the bony canal with the cord *in situ* was exsected for some distance above and below the point of section, placed in alcohol for twenty-four hours, and then transferred to a two per cent. solution of bichromate of ammonia. The cord after being hardened was imbedded in paraffin in a Zeiss



microtome, and sections were made about the point of previous division. The part of the cord showing the most extensive division was placed in carbolic acid and turpentine, and mounted in Canada balsam.

When a hemisection of the cord was made it was found that irritation of the medulla caused sweating in the foot opposite the side of section. Pl. III. Fig. 1 represents the hemisection. This conflicts with the statement of Adamkiewics, but he made a section in the lumbar region and then irritated the medulla, obtaining sweat in both extremities. His experiment would not exclude a transmission of impulses to the sweat glands by their fibres running in the abdominal sympathetics. Neither would it be surprising to find sweat appear in both extremities, as Woroschiloff has found that irritation of the medulla after a hemisection or more than a hemisection caused movements in the post-extremities. If the posterior columns are divided, as in Pl. III. Fig. 2, then sweat appears in both extremities as usual. If the anterior columns with a part of the lateral are divided (Fig. 3) and the medulla irritated, still sweat appears in both hind feet. If a great part of the lateral column on one side is divided and a smaller part on the opposite side (Fig. 4), then sweat will appear on the side where the lateral columns are not so much cut away, whilst none will be seen on the side of the extensive section.

If the gray matter is quite extensively divided (Fig. 5), still sweat ensues in both posterior extremities as though nothing had happened to the cord. If now the lateral columns are divided by a section through them, as in Fig. 6, not completely dividing them in their transverse diameter, no sweat will be seen to take place. The fact of sweat appearing only on the side opposite section would seem to demonstrate that the sweat-fibres do not decussate in the case of the animal operated on. These experiments demonstrate that the secretory fibres connecting the sweat-centres together run in the lateral columns of the cord, at least in the dorsal region of the cord about the 6th and 7th dorsal vertebræ.

**INHIBITORY FIBRES.** In another paper it has been shown that when the spinal cord is divided the sphincters of the anus and vagina set up a rhythmic movement. These movements are dependent on an ano-spinal and vagino-spinal centre seated in the spinal cord between the 6th and 7th lumbar vertebræ in the case of the cat. So when it is destroyed and the animal lives several days the sphincters remain perfectly quiescent, unlike the circular muscles in the arteries

which take on a rhythmic contraction and dilatation after division of their nerves. To discover the path of the impulses which inhibit the rhythmic action of the sphincters, the method employed was the same as that used in tracing the path of the secretory fibres. The sections were made between the 6th and 7th dorsal vertebræ. After the operation the animal was allowed to rest several hours, when the cord was excised as in studying the path of the sweat-fibres. When a hemisection of the cord was made, Fig. 1, no rhythm was set up. If the posterior columns are divided, Fig. 2, no rhythm is seen. If the anterior columns are divided, Fig. 3, no rhythm appears. If the lateral columns are divided, as in Fig. 4, no rhythm is seen. If however both lateral columns are divided, as in Fig. 6, then a rhythm is set up in a complete manner. The rhythm does not appear when the gray matter is divided, Fig. 5, but if now the cord is divided completely in the cervical region the rhythm appears. To determine the source of these inhibitory fibres in the cat's brain, I trephined from before backwards, and by a small spear-shaped knife made transverse sections through the brain. When a cut was made in the corpora striata dividing them on both sides in a transverse direction, the rhythm did not appear. If however a cut was made just between the corpora quadrigemina and the thalami optici down through the crura cerebri, then the rhythm came on. If the cerebrum be broken up the rhythm fails to appear. If a cut is made through the corpora quadrigemina and pons on the line just between the nates and testes, then the rhythm appears. These facts leave no doubt but that the thalami optici contain in part at least the centres of the inhibitory apparatus presiding over the ano-spinal and vagino-spinal centres. That additional co-operative centres do not exist in the corpora quadrigemina I am not prepared to state.

The sections figured represent but a small number of the experiments I have made on the subject.

The figures are considerably enlarged, being drawn from sections magnified by a glass.

*Fig. 1.*



*Fig. 2.*



*Fig. 3.*



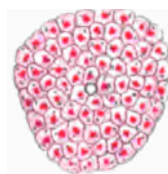
*Fig. 4.*



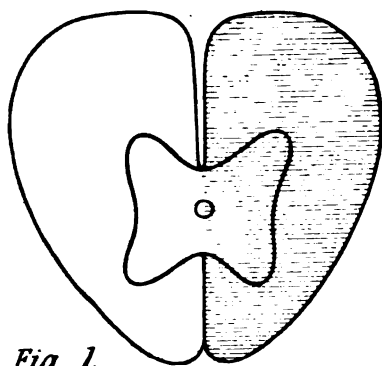
*Fig. 5.*



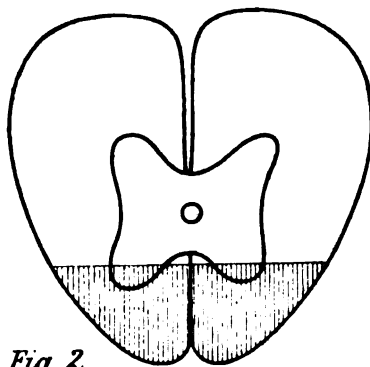
*Fig. 6.*



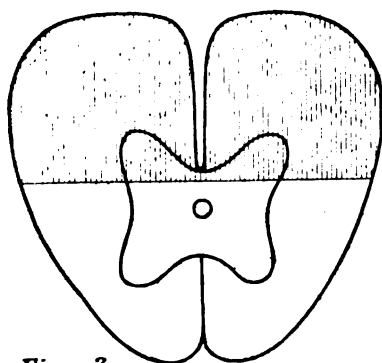




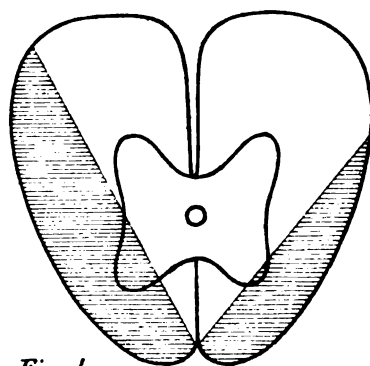
*Fig. 1.*



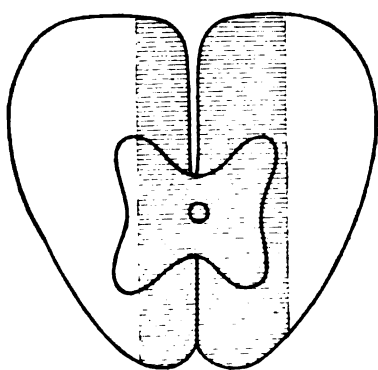
*Fig. 2.*



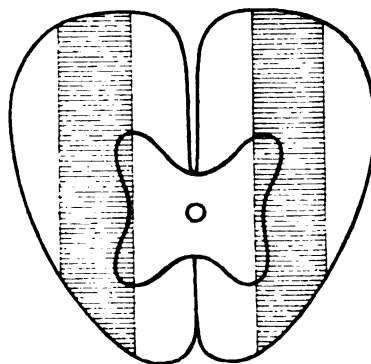
*Fig. 3.*



*Fig. 4.*



*Fig. 5.*



*Fig. 6.*



ON THE EFFECT OF TWO SUCCEEDING STIMULI  
UPON MUSCULAR CONTRACTION. By HENRY  
SEWALL, *Fellow of the Johns Hopkins University, Baltimore.*  
(Plate IV.)

THE attention of investigators of muscular contraction has, of late, been directed particularly to the limits of the frequency of stimulation within which a succession of stimuli must be included in order to produce a complete tetanus; and also to the alteration of the irritability of the muscle due to excitation.

Most of the study heretofore has been upon tetanus produced by many succeeding stimuli, but in the following pages the summation of two simple contractions only will be considered. The nature of the results of all such work must depend upon the character and laws of certain variables out of which a tetanus is built up; and it was the object of the research described in the following pages to elucidate the effect in the summated contraction due to them, of two single stimuli succeeding one another at various intervals of time, and to endeavour to get a clearer idea of the factors of that simple tetanus.

#### *Apparatus and method.*

The instrument employed was an elaborate form of the pendulum myographion of Fick, as modified by Mr Dew-Smith of Cambridge, and made by Elliot Bros. of St Martin's Lane, London. The working part of the apparatus is supported by a massive tablet of wood, which is screwed upon the wall of the work room. The pendulum is made up of a framework, some 43 inches long, of steel carried by an axle moving on friction wheels, and having at its lower end, before and behind, supporting clamps for two heavy glass plates. As in Fick's instrument, the tracing is recorded upon the front plate, the other serving merely as a compensating weight moving opposite to the first when this is screwed up or down by wheelwork carried within the base of the pendulum. An electro-magnet, movable on a graduated



arc behind the pendulum, may be made to hold or release the latter when desired, and swings of various amplitude can be obtained by sliding it to and fro upon the arc. A toothed spring carried by the pendulum is caught and held at the end of the swing by an eccentric wheel, which is also movable on the arc.

The pendulum carries below an insulated arm which in passing breaks the platinum contacts of two separate electric keys; these latter may each, by a micrometer screw, be moved along a graduated scale; thus two breaks may be had either simultaneously or succeeding each other at various intervals. In the experiments each key was interpolated in the primary circuit of a du Bois-Reymond induction coil, and these two coils were set at some distance from and at right angles to each other. A commutator was included in each primary circuit.

The wires from each secondary coil passed through an ordinary make and break key to the electrode clamps of a small moist chamber; the latter was placed upon a low table, which was movable by screws upon rollers in such a manner that the muscle lever might be moved up to or away from the recording plate without changing the relative positions of the moist chamber, tuning-fork, &c., carried on the table. The electrodes used were always "non-polarizable," a pair being in connection with each induction coil. The lever employed was a light Marey instrument, amplifying about 12 times, suspended by a thread and fastened to the tendon of the muscle by a light hooked needle. The stimulating battery was a single small carbon-bichromate cell for each induction coil. The muscle used was the gastrocnemius of the frog, with its attached sciatic nerve. The experiments occupied the months from October to January inclusive. The tracing was recorded on smoked glazed paper placed over the glass plate of the pendulum. A König tuning-fork giving 200 double vibrations a second wrote under the point of the muscle lever. The length of the pendulum made the abscissa line one of small curvature; and the unusual stability of the whole apparatus, with the nice adjustability of the subjacent contact keys, reduced mere mechanical errors and gave the power of determining small intervals of time with considerable accuracy. By closing either key and bringing the breaking arm of the pendulum into contact with it, the point of the muscle lever would then denote on the abscissa line the exact instant of the entrance of the stimulus into the nerve from the "break" of the primary circuit passing through that key. Thus in Pl. VI. Fig. 2 the point of the lever has

been used in this way to mark at *a* its position at the entrance of the stimulus giving rise to one single contraction, and at *b* its position when the stimulus for the other contraction was let into the nerve. The distance between this point and the commencement of the curve of the corresponding contraction measures, of course, the latent period of the muscle plus the time of transit of the impulse through the piece of nerve. The interval elapsing between the successive openings of the two keys, measured by marking in this way on the abscissa line the times of the entrance of the stimulus from each and drawing perpendiculars to the tuning-fork curve, I shall call the "interval of stimulation." All measurements were made with the compass and laid off on a millimetre scale. In each double stimulation the first shock entered the nerve from the pair of electrodes nearest the muscle, and the second from the pair farthest from the same; the distance between the two pairs of electrodes on the nerve was less than one centimetre, and, taking the rate of the nervous impulse at 28 metres per second, the interval of stimulation as calculated from the distance between the marks on the abscissa line is in error by about  $\frac{1}{3000}$  second. Breaking shocks alone were employed, and the pendulum struck both keys under practically the same momentum. Maximal stimuli were always chosen; in order to be sure of these, the secondary coils were moved just far enough away from the primary to avoid stimulations due to unipolar action which might cause contraction through breaks of the primary current, even though the secondary circuit should be open. The amplitude of the swing of the pendulum was made such that the beginning of the contraction due to the opening of one key should about coincide with the end of the contraction from the other, when the keys were separated as far as possible. The muscle was weighted with 20 or 30 grammes according to size. Sometimes an arrangement was used by which the lever was supported when it had reached the position which it would mark on the plate when the muscle was first fully stretched by the weight; a sort of "after-load" arrangement to prevent gradual stretching of the muscle under the influence of a continued load. Everything being in readiness, the experiment was begun either at the smallest or the greatest interval of stimulation. The summated contraction was first obtained, both keys being opened at one swing of the pendulum; and then, without changing the relative position of the keys, single contractions, first from one key and then from the other, were taken, all being recorded on the same base line.

The keys were then moved a definite distance to or from each other; the recording plate was screwed up or down to obtain a new base line, and the observations were repeated to the end of the experiment. After the observations the recording lever was made to trace a tangent to the summit of each curve for convenience in measurement as in Pl. VI. Fig. 2. Every experiment was thus recorded in a series of tracings like those in Figs. 1—3; the interval of stimulation alone being altered for each abscissa line. The direction of the current was changed at each stimulation to avoid polarizing the nerve. It was necessary to use the hand to move the pendulum back to the electromagnet which held it, and to close and open the circuit through this latter; the periods of rest between different contractions were therefore not of quite constant duration. There elapsed, on the average, between the stimulations giving rise to successive curves about 40 seconds. However unsatisfactory such conditions may appear, it seems that the many hundred observations made would tend to eliminate errors in the mean; and practically there appeared no evidence to show that different results could have been obtained by using the most exact intervals.

#### *Construction of the Table.*

In Table I. are shown the results of the series of observations in two selected from nearly a score of similar experiments. In the first column the interval elapsing between the entrance of the stimuli for the two single contractions whose succession has given rise to the tetanus on the same line in the third column, is given in  $\frac{1}{100}$  second. The heights of the single contractions operated on at different stages of the experiment are given in the second column in millimetres. In the third column is recorded the height of the double contraction curve due to the succession of two contractions, of height marked in the second column on the same line, at the interval in the first column. The increase in the height of the curve of the double or tetanic contraction over that of the single ones whose fusion makes the increase will be called simply the "Amount of Summation" of these contractions; as seen in Figs. 1 and 2, it is evidently the distance between the tangent to the curves of the single contractions and that of the tetanus. The amount of summation in millimetres for different intervals of stimulation is given in the fourth column. The remaining

columns show respectively the height in millimetres which the curve of the first contraction had attained at the entrance of the second stimulus, and the height of the first at the beginning of the second contraction curve.

Table I.

*Experiment 1.*

	(1)	(2)	(3)	(4)	(5)	(6)	
Number of Observation.	Difference between the times of stimulation in 0.01 seconds.	Greatest height of the single contraction in mm.	Height of the summated contraction in mm.	Amount of summation in mm.	Height of 1st contraction at entrance of 2nd stimulus.	Height of 1st contraction at beginning of 2nd contraction.	Remarks.
1	0.1	19.	19.9	0.9	0.	0.	Began at small interval. Load 30 grms. The 1st contraction lasted $\frac{7}{100}$ second.
2	0.15	19.5	27.2	7.7	0.	0.	
3	0.2	19.5	27.5	8.	0.	0.6	
4	0.3	19.4	27.9	8.5	0.	1.1	
5	0.4	19.4	27.4	8.	0.	1.7	
6	0.5	19.5	27.8	8.3	0.	1.8	
7	1.1	19.6	28.6	9.	0.	6.9	
8	1.7	19.6	29.8	10.2	1.3	11.8	
9	2.3	20.	31.2	11.2	5.3	14.6	
10	2.8	20.	32.6	12.6	10.1	18.	
11	3.4	20.	33.2	13.2	14.2	19.5	
12	3.9	20.1	34.1	14.	16.	19.8	
13	4.4	20.	34.4	14.4	18.3	19.4	
14	4.9	19.8	34.8	15.	19.6	17.1	
15	5.6	20.	34.8	14.8	20.	14.8	
16	6.	19.7	32.2	12.5	19.	10.4	
17	6.6	19.8	28.6	8.8	15.	4.	
18	7.	20.	23.9	3.9	11.	0.	
19	7.5	19.9	20.9	1.	5.2	0.	

*Experiment 2.*

	(1)	(2)	(3)	(4)	(5)	(6)	
Number of Observation.	Difference between the times of stimulation in 0.01 seconds.	Greatest height of the single contraction in mm.	Height of the summated contraction in mm.	Amount of summation in mm.	Height of 1st contraction at entrance of 2nd stimulus.	Height of 1st contraction at beginning of 2nd contraction.	Remarks.
1	8.1	18.4	19.1	0.7	2.6	0.	Began at the great interval.
2	7.5	18.6	19.4	0.8	5.1	0.	
3	7.	18.9	21.9	3.	9.	0.	
4	6.5	18.8	24.6	5.8	13.	2.8	After-load 30 grms.
5	5.9	19.	28.7	9.7	16.1	5.1	
6	5.4	18.9	31.3	12.4	18.8	11.6	The first contraction had ended in $\frac{1}{100}$ second.
7	4.9	18.7	32.6	13.9	18.5	15.8	
8	4.4	18.8	33.	14.2	18.	18.	
9	3.9	18.8	32.8	14.	16.2	18.8	
10	3.4	18.8	31.6	12.8	12.7	18.	
11	2.8	18.9	30.7	11.8	9.1	16.8	
12	2.2	18.8	28.9	10.1	5.1	13.2	
13	1.7	18.9	26.9	8.	1.8	10.8	
14	1.1	18.8	25.6	6.8	0.	6.	
15	0.6	19.	25.5	6.5	0.	3.	
16	0.4	19.	25.3	6.3	0.	1.8	
17	0.3	19.	25.1	6.1	0.	1.6	
18	0.2	19.	23.8	4.8	0.	0.8	
19	0.15	18.8	23.4	4.6	0.	0.6	
20	0.15	18.8	23.3	4.5	0.	0.5	
21	0.1	19.	20.4	1.4	0.	0.	
22	0.1	18.9	20.3	1.4	0.	0.	
23	0.05	19.	19.	0	0.	0.	

*General Results.*

A glance at the second column shows sometimes irregular variations in the height of the single contractions, these now being slightly higher, now lower; oscillations doubtless due to the shifting irritability of the muscle passing from a resting to a periodically excited condition, and which Kronecker<sup>1</sup> has found so prominent in the muscular reactions of the frog at certain seasons. More frequently the height of the single contraction continually diminishes owing to fatigue.

<sup>1</sup> *Sitzbericht. der K. Berlin. Akad.* 1870, p. 638.

Results of different experiments show that in separate muscles the ratio of the highest summation to the height of the single contraction, consequently the increased work done, varies somewhat; a result we should be led to expect from the quality differences of muscles.

The duration of the simple contractions of different muscles varies, but in any muscle the duration of the single contraction usually diminishes for some time after beginning work before passing into the progressive lengthening indicating fatigue. A warm muscle contracts more quickly than a cold one, and the heat developed in contraction is no doubt the cause of the primary lessening of the duration of the shortening.

### The Curve of Summations.

The smallest interval of stimulation of which account was taken was about  $\frac{1}{1000}$  second. If the preparation be very fresh a slight summation, or increase in the height of the tetanic over that of the single contraction, is seen even at this interval. As the interval between the stimuli producing the summated contraction is gradually increased, the amount of summation in the tetanic contraction shows first great irregularity, and then, as the intervals farther increase, the height of the summation gradually increases, attains a maximum at a definite interval of stimulation, and then more rapidly declines, as the intervals still increase. If the summated contractions could all have been recorded on the same abscissa, each at a distance from the origin corresponding to the interval of stimulation giving rise to it, the envelope of the tetanic curves would have a very irregular course at that part corresponding to the smallest intervals of stimulation; it would then rise gradually to a maximum, and then fall again more suddenly. In Fig. 4 is such an envelope constructed from experiment 2 by marking the heights of the summations in column 4 on ordinates meeting the abscissa at distances from the origin corresponding to the proper intervals.

One division on the abscissa = 0.002 second; one on the ordinate = 1 mm. At the smallest intervals on the left the summation begins by a sudden leap; then there comes a short period during which the height of the tetanus increases irregularly; then there is another decided increase in height corresponding to a greater increase in the interval of stimulation, and then the curve ascends pretty regularly to its maximum and then declines somewhat more quickly and

regularly. Near the end of its fall the "Summation Curve," as this may be called, evinces a tendency to become parallel to the abscissa line. The distance of any point on the base line from the left extremity of the latter marks the interval of stimulation at which the summation of two contractions (i.e. the increase over the curves of the single contractions) equals the height marked by the summation curve on the corresponding ordinate.

### *Effects of the First Stimulus.*

The length of the base included between the extremities of the summation curve represents an interval of 0.081 second. When the two stimuli had succeeded each other at this interval, the first contraction had already allowed the muscle to return to its position of rest when the second contraction began. Still, on breaking both keys at one swing of the pendulum, the second contraction so obtained is higher than either of the single ones obtained alone; and we have here evidence that the first contraction left the muscle in a condition such that it could respond more powerfully to a succeeding stimulus although the excitement causing the first contraction had passed off.

Inspection of Fig. 3 will illustrate this point. The result here obtained is at variance with the statement of Kronecker and Stirling<sup>1</sup>, that a muscular contraction may begin in so late a phase of a preceding one that no increase takes place in the height of the second. When one contraction succeeds another at the termination of the first, the height of the second curve must be influenced by the bounding of the lever due to the fall of the elastic weighted muscle after the first contraction; these movements of the lever would be more marked the more elastic the muscle. In some few cases I have found, as did the authors quoted, that, particularly in the fresh muscle, the second curve of the double contraction was less than or equal to that of the single one in height, when the curves of the single contractions barely met on the base line.

As is well known, at very small intervals of stimulation, the curve of the tetanic contraction begins with that of the first, and has a course parallel to that of either single one, differing only in its greater height and extent from that of the single contraction. Fig. 1 will perhaps recall the relation of the summated and single contraction curves at a small interval of stimulation. It is noteworthy that at the smallest

<sup>1</sup> *Journal of Physiology*, Vol. 1. p. 384.

intervals of stimulation the amount of summation is very much greater than the height which the first single contraction had attained at the beginning of the second. As the stimuli were maximal, and there was little or no mechanical lift preceding the second stimulus, we conclude that the muscle was put by the first stimulus into a condition modifying its reaction toward a succeeding one, giving in effect a result similar to that seen to be brought about in succeeding contractions not overlapping.

#### *Fusion of the Tetanus Curve.*

As the interval of stimulation increases from its smallest value, the height and extent of the summated curve becomes greater, and the once continuous curve begins to show a sinuation, indicating the superposition of one contraction upon another. The point at about which the complete fusion of the tetanus curve disappears, and at which its compound nature is first manifested, is very constant. It is at a mean interval of .026 second, corresponding to 38.8 stimuli a second.

#### *The greatest Summation.*

The relative position at which the succession of two contractions gives the greatest summation coincides with two things. The greatest summation occurs when the beginning of the second contraction curve falls upon the highest point attained by the first; here is the greatest mechanical advantage which the first contraction can afford. But there is reason to think that the interval at which the stimuli succeed each other is of great importance apart from that; this interval for the point of greatest summation is tolerably constant; the mean of the tabulated experiments gives it as 0.048 second, corresponding to 20.8 stimuli per second. This is about the frequency of stimulation with which a frog's gastrocnemius may just be held in continuous tetanus.

#### *Rate of Stimulation.*

*A priori* we might expect that succession of stimuli which should call forth the greatest work power with least expenditure of energy to be used in the body itself.

This best relation of force to work seems to be possessed by the number 20.8 stimuli per second in the case of the frog's gastrocnemius, and suggests the frequency of stimulation as determined from the muscle-note in the human subject. It seems not improbable that the rate of stimulation capable of eliciting the greatest sum total of work



from the frog's gastrocnemius, and therefore the rate most favourable to the maintenance of the irritability of the muscle, should be this of 20·8 stimuli in a second. It seems settled that the more frequently the stimulations succeed each other, to a certain extent, when an excised muscle is tetanised, the higher is the contraction curve. This fact does not weaken the suggestion made above, for it is presumable that the more frequent the stimulation the less is the sum of the mechanical work done in proportion to the energy evolved; for Kronecker<sup>1</sup> found that the more rapid the succession of stimuli producing tetanus the more steeply fell the fatigue-curve.

Whether, however, the statement of Marey<sup>2</sup> is correct, that in a voluntary contraction the frequency of stimulation is greater the more powerful the shortening, is not yet certain: the proof given is the elevation in the pitch of the muscle-note from the masseters with increased energy of contraction.

The observations made above, and the result of the inspection of many series of curves, conform with the statement of Helmholtz<sup>3</sup>, that from two succeeding stimuli the contraction due to the second does not add itself to the first until the natural latent period of the second is past; the second contraction begins at the same time whether taken alone or fused with a preceding one.

However, we shall meet conditions in which this does not appear to be true.

#### *Smallest interval giving a Summation.*

As before observed, the smallest interval of stimulation of which note was taken was about  $\frac{1}{1000}$  second. At this interval, if the preparation were quite fresh, there was a decided summation of contractions at the double stimulation. To get a summation at this interval it was usually necessary to begin the experiment with the rapidly succeeding stimuli; if the experiment began with contractions obtained at great intervals, the stimulating keys being gradually approached, the increase in the summated over the single contraction almost uniformly ceased at an interval of  $\frac{1}{300}$  to  $\frac{1}{200}$  second.

Proceeding definitely to investigate this point it was found that a muscle in a fresh or after a resting condition, stimulated by induction shocks through its nerve, would give a summation

<sup>1</sup> "Ermüdung u. Erholung," &c. Ludwig's *Arbeiten*, 1871, p. 177.

<sup>2</sup> Marey, *Mouvement dans les fonctions de la vie*, p. 455.

<sup>3</sup> *Monatsbericht. zu der Berlin. Akad.* 1851, p. 328.

in the double contraction at very small intervals of stimulation. But continued work would very quickly cause the summation to fail at the smallest intervals. If the keys were then separated somewhat, there was again a summation at the new interval; continued work caused the increase to fail here, to again return on increasing the interval. A failure of the summation of the *double stimuli may thus in certain conditions occur when they succeed each other at an interval of  $\frac{1}{100}$  second or more*. After resting sometime, however, the muscle will again give an increase in the summated contraction at the small intervals of stimulation, and at a smaller interval the longer the rest.

In all cases when the summation with the double stimulation failed, the curve of the actual contraction coincided with that of the simple one due to the opening of the first key. The result cannot be due to magnetic influences in the cores of the inductive coils, as might be the case in a continued tetanus with a du Bois apparatus<sup>1</sup>, for each induction coil, with its myograph key and stimulating electrodes, was altogether separate. The phenomenon appeared to bear no definite relation to the direction of the stimulating current. The interval of stimulation at which the failure of the summation usually occurs is strikingly near that with which Bernstein<sup>2</sup> got only an "initial contraction instead of continued tetanus;" but the results here obviously are not to be explained on Bernstein's hypothesis of an interference of impulses within the muscle. If the phenomenon be of functional importance it may be described as a fatigue of the muscular tissue respecting quickly succeeding stimuli.

It will be noticed that the result here obtained is not necessarily opposed to that obtained by Kronecker and Stirling<sup>3</sup>, who got from shocks induced by the longitudinal vibrations of a rod a perfect tetanus, giving rise to the conclusion that the upper limit of the frequency of stimulation at which a tetanus fails must exceed 20,000 per second. In the experiments of these workers the succession of stimuli was kept up upon a muscle in different stages of contraction; whereas in my experiment the first contraction had not at all or but just commenced when the second began, and was met by this in but a single phase of its excitement. This question will be considered further, below.

The work of Valentin<sup>4</sup>, as far as I can understand it, on the effects

<sup>1</sup> Marey, *Mouvement dans les fonctions de la vie*, p. 382.

<sup>2</sup> *Nervensystem*, p. 113.

<sup>3</sup> *Loc. cit.*

<sup>4</sup> Pfüger's *Archiv*, Bd. vii. p. 453.

of succeeding stimulations with the constant current on muscle-nerve preparations, seems to include results somewhat analogous to the above.

The familiar work of Helmholtz<sup>1</sup> on the summation of two succeeding contractions gives the smallest interval at which two maximal stimuli succeeding each other can give a summation of the contraction as  $\frac{1}{800}$  second. But, while considering the errors of my method of determination, it will be seen from inspection of the figures that it would not be difficult to estimate a distance equal to less than  $\frac{1}{4}$  of the space from crest to crest of the tuning-fork curve, which fraction corresponds to  $\frac{1}{1000}$  second; this matter will be reconsidered farther on.

#### *The rate of descent of the Tetanic Curve.*

There is a peculiarity brought out by the study of a series of contractions such as are recorded in the tables. When, at the smaller intervals of stimulation, the summation of the double contraction becomes marked, its curve falls from its highest point in a time proportional to that of the fall of the curve of either single contraction; the two are parallel in their descent, see Fig. 1. As the interval of stimulation is increased, however, the descent of the tetanic curve becomes gradually relatively quicker than that of the single contraction; the fall of the former is steeper than that of the latter.

This is the most marked at the highest summations obtained, diminishing as the second contraction begins in a later phase of the first; see Figs. 2, and 3. It will be shown that mere increase in the height of a contraction does not necessarily alter the rate of descent of the muscle curve. An explanation of the fact may be had if we presume that in the summation of two contractions at a favourable interval of stimulation the contractile energy is condensed and used up rapidly in doing work, leaving the muscle an inert body to be rapidly stretched as such by the suspended weight when the shortening phase of its contraction is over; while in a single contraction, the contracting energy is not expended at the greatest shortening, but resists for a time the fall of the appended load.

#### *The influence upon Muscular Contractility of preceding Stimulation.*

There is a peculiarity due to the activity of muscle which has probably been observed by all experimenters upon this tissue, and which is usually expressed as the increase of irritability of the muscle, or per-

<sup>1</sup> *Loc. cit.*

haps the nerve, due to stimulation. Fick<sup>1</sup> found that the work power of a muscle was increased by a limited activity; Minot<sup>2</sup> that the increase of irritability, as shown in the height of contraction with given load, is modified by definite conditions and has a determinate course.

I noticed that at very small intervals of stimulation the tetanic curve rose considerably above that of the single contraction when there could be little mechanical advantage due to the first contraction, Fig. 1. Again, when the stimulating keys were so far separated that the single contractions did not overlap, there was still an increase in the height of the second of the double contraction curves, Fig. 3. We have in both cases the same result, that a stimulus puts the muscle into a condition which modifies its reaction toward a succeeding stimulus.

With the desire of studying this point, the electro-magnet was placed upon its arc on the myograph so as to allow only a slow swing of the pendulum, so that when the keys beneath were separated as far as possible, the single contractions from each should be by a considerable interval on the base line.

A small electro-magnet with soft iron core was obtained, and a soft iron disk serving as its armature was made fast to the muscle lever, and the thread upon which the muscle pulled was fastened to the armature itself in such a manner as not to distort the lever during contraction. A carbon bichromate-cell was used for the magnet, and the wires of the latter were clamped upon one of the stimulating keys, that key which should be opened last in the swing of the pendulum. When this key was closed the battery circuit passed round the magnet, and the armature was held firmly against its core, and the lever could not be raised; when the key was opened, things were so arranged that the magnetism of the core should be lost.

Separating now the keys as far as possible, a contraction was obtained from the first without using the magnet. The pendulum was then replaced, the second key was closed (thus completing the magnetic as well as the stimulating circuit), and a contraction was obtained from this key alone. This second contraction was usually somewhat higher than the first for the magnet, not being of soft enough metal, lost its magnetism gradually, causing a slight resistance to the contraction, thus increasing its power. Knowing now the interval of stimulation and the form and amplitude of the contraction curves, both keys were closed to be opened by one swing

<sup>1</sup> *Untersuch. ü. Muskelarbeit*, p. 9.

<sup>2</sup> Experiments on Tetanus, *Journal of Anat. and Physiology*, Vol. XII. 1878, p. 237.

of the pendulum. The stimulation due to the opening of the first key was powerless to cause the muscle to shorten since it could not overcome the attraction of the electro-magnet; its excitement was passed without disturbing the position of the lever upon the base line. As the pendulum proceeded in its swing the second key was opened in turn and a contraction obtained whose curve began at the same point where that of the trial contraction from the same key had began. The stimulating keys were then gradually approached and the process repeated at various intervals of stimulation.

It was found that a given maximal stimulus stirs up the untired muscle to a more powerful contraction when it has been preceded by the excitement ordinarily producing contraction.

This increase of contractility due to stimulation is apparently a character of the fresh muscle; it fades away gradually and disappears first at the greatest intervals of stimulation; a curve representing its force would be highest at the period of the greatest energy of the excitement due to the first stimulation.

It seems certain, indeed, that on continued work, this influence of the first stimulus changes its sign and leaves the muscle in a less irritable condition as regards succeeding stimuli. But it was impossible with the myograph to make use of sufficiently definite periods between the different sets of observations in an experiment to undertake this question. In those rather rare instances in which the curve due to the first of two succeeding normal unrestrained contractions had coincided with the base line before the beginning of the second, this contraction curve was invariably higher than the first. If a single contraction immediately precede, but be not included within the tetanic contraction due to breaking both contacts at one swing of the pendulum, the tetanic curve in this case would be much higher and more continuous in direction than normally. A number of accidental occurrences having this result led to the study of the influence upon the irritability of the muscle exerted in general by tetanic stimuli. In the experiment, a wire was made fast to the first key of the myograph so that it should be lifted out of a mercury cup, and break the circuit in a third induction coil a period after the opening of the first key. The secondary wires of this last coil were attached one to either of the original pairs of stimulating electrodes. Barely opening the first key now gave the ordinary stimulus from it; but on moving the key still farther from its contact, the wire bound to it was lifted out of the mercury cup and the

primary circuit of the third induction coil was broken. Thus the pendulum in its swing would move the first key so far that two successive stimuli should come from the opening of the one key. The tetanic shortening excited by opening the first key was restrained by the means used for the first single contraction in the preceding experiments. Proceeding in the same manner, now, as before, it was found that a tetanic stimulus, the excitement due to which has not been allowed to shorten the muscle, leaves the muscle in a state of greater irritability toward succeeding stimuli than does a single stimulus under the same circumstances; moreover the influence of the tetanic stimulation lasts longer than that of the single one.

This result appears to have an interesting relation to the apparent want of agreement of the fatigue curves as obtained by Kronecker<sup>1</sup> and by Minot<sup>2</sup>. The former used a succession of single stimuli and found that, after slight irregularity, the envelope of the contraction curves fell as a straight line. The latter worker applied tetanic impulses for some 4 seconds at intervals of 20 to 30 seconds, and found that the "Curve of Exhaustion begins with a rise which marks the time of increasing irritability."

Whatever opposition there may appear to be between the two conclusions is explained if we take into account the greater intensity and duration of the increase of irritability due to a tetanic stimulus as compared with that giving rise to a single twitch of the muscle.

#### *The Latent Period.*

If, instead of restraining altogether the single contraction due to the opening of the first key, the magnetic current be broken in a late phase of the first contraction, the muscle lever will be raised very slightly, shewing a remnant of the contracting power set up by the first stimulus. If now, from the same swing of the pendulum, a contraction from the second key succeed this remnant of the first at such an interval that the latent period of the normal second contraction shall overlap the beginning of the curve of the partially restrained first contraction, then the summated effects of the two contractions is much greater than that of either alone; and this greater curve begins at the point at which the tiny curve of the restrained first contraction begins. In other words, the latent period of the second contraction is nearly or

<sup>1</sup> "Ermüdung u. Erholung d. quergestreiften Muskeln," Ludwig's *Arbeiten*, 1871, p. 177.

<sup>2</sup> *Loc. cit.*

quite annulled, and the energy of the two contractions is summated at the entrance of the stimulus for the second.

This is a conclusion drawn from the inspection of numerous tracings; if there be no error involved, it exhibits an interesting relation between the processes going on during the latent period of stimulation and the tensions set up in the muscle by preceding stimuli, or rather by the restrained effort to contract.

It was mentioned early in this Article that in an ordinary tetanus no stimulus succeeding another showed its effect upon the contraction curve from this until the usual latent period of the second stimulus had elapsed.

#### *Contractions from Artificially Shortened Muscle.*

An attempt was made to determine the effect which the mere physical condition of shortening due to stimulation has upon succeeding contractions. If the biceps, for example, be made to raise the forearm and this then be supported by some external object in that position, the muscle may be said to have a new natural form in a different sense from that of Weber<sup>1</sup>; it now does no work and is in a resting state, but can succeeding contractions take advantage of this artificial shortening as of the active contractions of tetanus? A slow swing of the pendulum was employed as in the last experiment, and the stimulation keys were separated as far as possible. There was attached to the recording lever, and swinging freely by a hinge joint, a small brass rod with a bevelled end. On raising the lever the extremity of the rod moved lightly upon the inclined rim of a metal plate with a notch into which the end of the little rod could be dropped at any desired height, thus keeping the lever supported in an elevated position without weighting the muscle. Most of the observations were made upon muscles suspended vertically in the usual way. An ordinary contraction was first recorded from the break of each key; then the lever was supported in the notch of the brass plate, the muscle thus being unloaded and looped a bit, and a double stimulus was given at such an interval that the first contraction had already disappeared some time before the second stimulus reached the muscle, the first being used simply to bring the muscle into the form natural at that stage of contraction to which its extremity had been elevated.

It was evident from the outcome that the height of a contraction beginning from an artificially shortened condition is relatively less than

<sup>1</sup> Wagner's *Handwörterbuch d. Physiologie*, Bd. iii. p. 110.

that commencing from the full length of the muscle; the greater the artificial shortening, the less is the height of the contraction proceeding from it compared to that from the normal length of the muscle. This is of course what might have been predicted; for the less the length of muscle to start with, the less can be the extent of its shortening: but the advantage which the artificial shortening gives to a succeeding contraction is very much less than that due to an active shortening during which the muscle is a condition of considerable tension. The duration of each second artificially aided contraction was less in proportion to the height from which it began; and of two such succeeding contractions the height of the second was always the greater.

Considerable absolute increase over the height of the normal simple contraction may be had by this method. There may even sometimes be a slight contraction superadded when the preliminary shortening has been made to exceed that due to a normal simple contraction.

If the support under the lever be suddenly removed after the second contraction has begun, the fall of the curve will be parallel to that of the normal contraction, provided the contraction had not begun from a height greater than that which the normal would have attained; otherwise the fall is steeper than that of the normal contraction.

Another arrangement was made by fastening the whole leg of a frog in such a manner that the gastrocnemius should lie horizontally and draw the thread attached to its tendon over a pulley, the tendon alone being cut free for the purpose. Proceeding in the same manner as before, contractions from the artificially shortened muscle show at first a slight absolute increase in height over the normal contraction, and afterward an absolute diminution compared to it.

These last results are much more decisive than those usually obtained from the vertically suspended muscle in their support to the conclusion drawn from the experiment. The superiority of the latter method is evident, but the apparatus was not favourable to experiment on muscles in a horizontal position and few such were attempted. It will be noticed that in these experiments it was assumed that the internal distribution of the muscle molecules giving rise to the contraction form might persist for a period after the cessation of the functional excitement, when the muscle carried no weight; that the tensions accompanying any stage of shortening still determined the physical condition of a muscle in a form natural at that stage, whose activity had ceased. We should then be able to treat the muscle as a body whose extensibility had been vastly increased and its elasticity



proportionately diminished. The condition may be simulated by supposing a steel spring in a form due to great tension to be suddenly converted into soft iron. Weber<sup>1</sup> describes the cessation of active contraction of a muscle, as observed through the microscope, to be accompanied by a bending and looping of its fibres. This fact would, partially at least, make untenable the assumption advanced above; still it does not alter the main result, that the greater the preliminary shortening, and therefore the more extensible the muscle to begin with, the less is the contractile or work-power educed by a succeeding stimulus.

The results on the supposition of the change of physical properties alone in the artificially shortened muscle also satisfy Weber's<sup>2</sup> statement, that the differences in the work-power of muscles lies in the variability of their elastic coefficients.

Heidenhain<sup>3</sup> has made out that the kinetic energy set free in contraction is a function of the tension within the muscle at the beginning of and during contraction. This function, at least its mechanical element, has been here found to increase with the length, but only as this increase was attended with a diminution of extensibility; it follows then that, considering this variable, the most work can be done by a muscle the greater its length at the beginning of contraction; provided the preliminary stretching does not diminish the elasticity of the muscle. In regard to this last Fick<sup>4</sup>, who has worked out the above conclusion with thoroughness in other ways, has found that the frog's gastrocnemius is capable of doing the greatest total of work in contraction with a load of from 50 to 70 grms.; Marey<sup>5</sup> states that the load which first permanently stretches the same muscle, and therefore alters its elastic coefficients, is in the neighbourhood of 50 grms.

#### *The Rate of Liberation of Energy in a Single Contraction.*

Fick<sup>6</sup> originated an interesting experiment to the purpose of finding out the rate of development of energy at different stages of a single contraction. Thanks to a good instrument I was able to obtain, perhaps, more definite results on this point.

A rapid motion of the pendulum was used.

The first key of the myograph alone was employed in stimulating; the electro-magnet under the recording lever being in connection with

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<sup>3</sup> *Mouvement dans les fonctions de la vie*, p. 301.

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that commencing from the full length of the muscle; the greater the artificial shortening, the less is the height of the contraction proceeding from it compared to that from the normal length of the muscle. This is of course what might have been predicted; for the less the length of muscle to start with, the less can be the extent of its shortening: but the advantage which the artificial shortening gives to a succeeding contraction is very much less than that due to an active shortening during which the muscle is a condition of considerable tension. The duration of each second artificially aided contraction was less in proportion to the height from which it began; and of two such succeeding contractions the height of the second was always the greater.

Considerable absolute increase over the height of the normal simple contraction may be had by this method. There may even sometimes be a slight contraction superadded when the preliminary shortening has been made to exceed that due to a normal simple contraction.

If the support under the lever be suddenly removed after the second contraction has begun, the fall of the curve will be parallel to that of the normal contraction, provided the contraction had not begun from a height greater than that which the normal would have attained; otherwise the fall is steeper than that of the normal contraction.

Another arrangement was made by fastening the whole leg of a frog in such a manner that the gastrocnemius should lie horizontally and draw the thread attached to its tendon over a pulley, the tendon alone being cut free for the purpose. Proceeding in the same manner as before, contractions from the artificially shortened muscle show at first a slight absolute increase in height over the normal contraction, and afterward an absolute diminution compared to it.

These last results are much more decisive than those usually obtained from the vertically suspended muscle in their support to the conclusion drawn from the experiment. The superiority of the latter method is evident, but the apparatus was not favourable to experiment on muscles in a horizontal position and few such were attempted. It will be noticed that in these experiments it was assumed that the internal distribution of the muscle molecules giving rise to the contraction form might persist for a period after the cessation of the functional excitement, when the muscle carried no weight; that the tensions accompanying any stage of shortening still determined the physical condition of a muscle in a form natural at that stage, whose activity had ceased. We should then be able to treat the muscle as a body whose extensibility had been vastly increased and its elasticity

proportionately diminished. The condition may be simulated by supposing a steel spring in a form due to great tension to be suddenly converted into soft iron. Weber<sup>1</sup> describes the cessation of active contraction of a muscle, as observed through the microscope, to be accompanied by a bending and looping of its fibres. This fact would, partially at least, make untenable the assumption advanced above; still it does not alter the main result, that the greater the preliminary shortening, and therefore the more extensible the muscle to begin with, the less is the contractile or work-power educed by a succeeding stimulus.

The results on the supposition of the change of physical properties alone in the artificially shortened muscle also satisfy Weber's<sup>2</sup> statement, that the differences in the work-power of muscles lies in the variability of their elastic coefficients.

Heidenhain<sup>3</sup> has made out that the kinetic energy set free in contraction is a function of the tension within the muscle at the beginning of and during contraction. This function, at least its mechanical element, has been here found to increase with the length, but only as this increase was attended with a diminution of extensibility; it follows then that, considering this variable, the most work can be done by a muscle the greater its length at the beginning of contraction; provided the preliminary stretching does not diminish the elasticity of the muscle. In regard to this last Fick<sup>4</sup>, who has worked out the above conclusion with thoroughness in other ways, has found that the frog's gastrocnemius is capable of doing the greatest total of work in contraction with a load of from 50 to 70 grms.; Marey<sup>5</sup> states that the load which first permanently stretches the same muscle, and therefore alters its elastic coefficients, is in the neighbourhood of 50 grms.

#### *The Rate of Liberation of Energy in a Single Contraction.*

Fick<sup>6</sup> originated an interesting experiment to the purpose of finding out the rate of development of energy at different stages of a single contraction. Thanks to a good instrument I was able to obtain, perhaps, more definite results on this point.

A rapid motion of the pendulum was used.

The first key of the myograph alone was employed in stimulating; the electro-magnet under the recording lever being in connection with

<sup>1</sup> Wagner's *Handwörterbuch*, Bd. III. p. 57.

<sup>2</sup> *Mechanische Leistung*, &c. pp. 90, 103, 107.

<sup>3</sup> *Mouvement dans les fonctions de la vie*, p. 301.

<sup>4</sup> *Op. cit.* p. 116.

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<sup>6</sup> *Muskelarbeit*, p. 56.

the second key. In the experiment a normal contraction was first obtained, both keys being opened simultaneously. The keys were then separated by a short distance and were again opened at one swing, the shortening due to the first key not manifesting itself of course on the tracing until the second key had been opened and the magnet demagnetized. Exact time relations were determined by means of the vibrating fork.

It was found, as Fick had established, that restraining a muscle from shortening after contraction should normally have set in, may greatly increase the contractile force of the muscle when let free.

This development of energy, as shown in the height to which the lever was thrown when released by the magnet, increases rapidly to a maximum and then diminishes more slowly.

The time elapsing between the stimulation and the greatest development of energy, as indicated by the greatest shortening, is not quite constant for different muscles, but always considerably precedes the period of maximum of the normal contraction; Fig. 5.

The functional changes efficient to cause a contraction fade away at quite different rates, even in the same muscle; and have probably always disappeared in a less time than that which would have been occupied by a normal contraction. This is in opposition to Heidenhain's<sup>1</sup> deduction that the liberation of energy continues throughout the period of the relaxation of the muscle. The restrained muscle contracts instantly on being let free; there is an apparent latent period preliminary to the shortening, but when a stretched rubber band was substituted for the muscle a latent period of about equal length was obtained, giving rise to the conclusion that it was only the effect of a residuum of magnetism in the core of the magnet after breaking its circuit.

The curve of the restrained contraction, when this is near its maximum, rises much more suddenly than that of the normal; its height may be more than double that of the latter, and its fall may or may not be parallel to that of the ordinary contraction. Inserted here is a Table from three out of a number of series of observations. In the first column the period during which the normal contraction has been restrained is given in  $\frac{1}{100}$  seconds. In the second column is the height which the curve of the normal contraction would have attained in this time, in millimetres, as measured from an actual contraction curve. In the third column is given the height in millimetres to which the lever

<sup>1</sup> *Mechanische Leistung*, p. 168.

was thrown when let go after this interval. In the fourth and fifth columns are shown respectively the duration and maximal height of the normal contraction tracing.

Table II.

*Experiment A.*

(1)	(2)	(3)	(4)	(5)
Time difference between the beginning of the normal and of the restrained contraction in $\frac{1}{100}$ secs.	Height of the normal contraction at the beginning of the restrained in mm.	Height of the restrained contraction in mm.	Duration of the normal contraction in $\frac{1}{100}$ secs.	Maximal height of the normal contraction in mm.
0.6	3.7	20.5	7.2	17
1.4	10.	28.6		
2.	14.	31.		
2.5	16.2	28.4		
3.5	16.8	15.2		
4.3	15.5	5.		
5.4	9.5	1.		
		0		

*Experiment B.*

0.6	4.2	29.2	9.	25.4
1.6	14.	37.5		
2.6	22.2	36.3		
3.6	25.2	35.6		
4.6	25.3	29.2		
5.6	22.8	10.5		
6.6	17.8	8.		
		0		

*Experiment C.*

0.9	5.5	32.5	9.7	26.
1.9	15.2	38.3		
3.	22.	34.2		
3.5	24.7	14.5		
4.2	26.	2.6		
5.5	24.	1.3		
		0		

B and C of the Table are two experiments from the same muscle.

If, as Fick supposes, the time relations of the evolution of energy in the restrained contraction are proportional to those of the normal

contraction, we have here some intimation of the variation of the tension changes during a single contraction, and a graphic may be constructed showing them. Fig. 5 is such from the series under A in the Table. The abscissa units correspond each to  $\frac{1}{100}$  second, and each ordinate unit to 2 mm. in the height of the contraction curve. The lower curve is described through points placed at the height which the normal contraction would have attained (as determined by actual measurement) when the restrained contraction, the height of whose curve is marked on the same ordinate, had first begun.

The restraining action of the magnet may be considered as the influence of a great load or "after-load" upon the muscle. We can determine the possible duration to which the latent period of stimulation may be extended by the greatest load which the muscle can lift; the contraction must begin, under these circumstances, at the period of the greatest liberation of energy. Taking the ordinary latent period as .01 second, we find from the mean of the above three experiments that the greatest shortening of the restrained muscle, and presumably its greatest contractility, occurred at the end of .051 seconds from the moment of stimulation; or .041 seconds after the cessation of the normal latent period. Conversely, knowing this period, the greatest lifting power which the muscle can exert may be measured by the weight which can just be raised when the contraction has been restrained until this period.

Curves constructed like that of the higher of Fig. 5 seem to be pretty constant in the proportions of the ascending limb; but the form of descent varies greatly.

#### *The Nature of Muscular Irritability.*

When one uses the term "Irritability" as a property of muscular tissue there is meant, in general, an expression of the power of reacting mechanically to varied stimuli, to which purpose the muscle has been specialized. The single word "irritability" is so far unfortunate in that it includes two characters of different significance, and better distinguished in the German expressions "Erregbarkeit" and "Leistungsfähigkeit," which, as regards muscle, we may define as Excitability and Contractility. A muscular contraction is set up, we may presume, only when an access of energy from without has disturbed a certain structural equilibrium in its molecules; the less stable this equilibrium is the less need be the force necessary to bring about disarrangement preliminary to contraction; i.e. the excitability of the

muscle to stimuli increases with the ease with which the equilibrium of the muscle molecule is disturbed. Again, if with Ranke we consider the final efficient stimulus to be the presence of a certain amount of some chemical substance in the muscle, then it might be that if a definite, but not sufficient, amount of this substance were already present, a less external stimulus than usual would be able to excite the muscle: having less of the ultimate chemical stimulant to form. These are qualitative characters of any given muscle.

On the other hand we have a quantitative relation to consider, the Contractility of the muscle, which is an expression for the amount of energy evolved in contraction.

This depends upon the amount of chemical changes occurring in a given time: and probably on conditions of the muscle in which a smaller or larger proportion of the energy liberated tends to take the form of mechanical work. Thus, according to Ranke<sup>1</sup>, the presence of certain substances, as kreatin and lactic acid, in the muscle increase its excitability toward minimal stimuli. If these matters are present in small quantity the contractility or work-power of the muscle is also increased, but if they be present in excess the work-power of the muscle is diminished, while its excitability to minimal stimuli is still greater than that of the normal resting muscle. Thus, too, after the injection of dilute nitric acid<sup>2</sup> the excitability of the muscle toward minimal stimuli is greatly heightened.

We know little of the conditions determining variations of excitability of the muscle, nor of the limits to which it is subject.

On the other hand it is pretty certain that the contractile power of the muscle depends upon the elastic tension existing between its molecules, and increases with it. Every series of contractions probably involves variations of both the factors of irritability.

Ranke's<sup>3</sup> view of the question is that during stimulation the natural decomposition processes of the muscle are hastened, and the kreatin and lactic acid appear in such quantities as not to be removed or neutralized by the blood, as in the resting state.

These substances themselves are the stimuli which immediately excite the muscle; and as a slightly acid medium favors the fermentation which produces lactic acid, this latter is formed at an increasing rate for a while; this increase in the quantity of the stimulating matter is the cause of the increased contractions and work done by the muscle for a period early in its activity. The acid reaction of the muscle now

<sup>1</sup> *Tetanus*, p. 348.

<sup>2</sup> *Ibid.* p. 399.

<sup>3</sup> *Ibid.* p. 450.

becomes so strong that further development of lactic acid is hindered, a strong acid reaction retarding its production.

A new reaction manifests itself in the kreatin and lactic acid at this stage; these substances divert to themselves the oxidative processes from the muscle molecule, whose decomposition is the precursor of contraction; this is the condition of fatigue.

According to this view the excitability of the muscle to stimuli increases with the quantity of lactic acid, and its contractility or work-power first increases and then decreases with it.

All the functional phenomena of muscle must finally depend upon chemical changes; but it is not clear how, on Ranke's theory, a fatigued muscle removed from the body recovers power during rest. Nor why, if contraction depends upon the preformation and is aroused by the presence of lactic acid, the chemical transformations in the muscle and work done vary with the load upon it; for all that has been changed in this case is the resistance to that manifestation of kinetic energy which it is the final purpose of the muscle to develop; if this energy were partly used to produce new stimulating materials, we might expect every momentary stimulus either to cause the muscle to contract to exhaustion, or to persist with diminishing intensity for an infinite time.

Under the definition of the terms employed here excitability has only to do with the variation in the difficulty of setting off the chemical changes producing contraction, and contractility with the amount of these or the proportion of them which take the form of mechanical work. Apparently the former factor is only to be considered in the use of submaximal stimuli. With maximal stimuli and constant load the variations in the work-power of the muscle might be due to variations in the strength of the impulse transmitted by the nerves, or to more or less capable conditions of the muscle itself. But, as to the first case, according to Helmholtz<sup>1</sup> and to Marey's<sup>2</sup> figures, decreasing the conductivity of the nerve by cold does not alter the height of contraction; and with maximal stimuli excitement of all parts of the nerve give the same amounts of contractions<sup>3</sup>. Therefore it seems that with maximal stimuli the strength of the impulse transmitted from the nerve to the muscle may be considered a constant, and that the condition of the muscle itself determines the energy to be evolved in any contraction.

<sup>1</sup> Müller's *Archiv*, 1850, p. 276.

<sup>2</sup> *Mouvement dans les fonctions de la vie*, p. 349.

<sup>3</sup> Helmholtz, *loc. cit.*

Aside from experimental evidence we should be led, on *a priori* grounds, to suppose that the marked physical characters of muscular tissue would have a very important economic relation to its functional activity.

It seems probable that the phenomena known as the variation in the irritability of the muscle, which I have tried to differentiate as its contractility, its increase for a period during the stimulation of the fresh muscle, and the decrease during fatigue, is a quantity whose value is determined by the physical condition of the muscle at any moment. Different muscles and the same muscle at different times vary greatly in the relation of their elastic and extensible properties. According to Weber<sup>1</sup> the work-power of a given weight of muscle with a given stimulus increases with the elasticity of the muscle, and decreases with its extensibility. Continued work increases the extensibility<sup>2</sup>; that is, the effect of stimulation persists to some extent in the muscle after the cessation of functional excitement, modifying its reaction to succeeding stimuli. According to Heidenhain<sup>3</sup> the amount of kinetic energy developed in contraction, a direct function of the chemical changes occurring, increases with the tensions in the tissue up to certain limit and then decreases with their farther increase. Fick<sup>4</sup> found the work done to increase up to a certain load and then diminish as the load farther increased. This last agrees with the rest if we suppose the greater loads to stretch the muscle to such an extent as to diminish markedly its elastic properties, as was found probable above.

In the experiment dealing with artificially shortened muscle, the second contraction was higher than the first; the first was due to the stimulation of a flaccid muscle, the second took place in the muscle in which it is fair to assume there was some increase of tension due to the previous contraction.

If we suppose then that, for a time, stimulation of a muscle increases its elasticity, and finally progressively diminishes it, the subject of the change of contractility of the muscle due to stimulation becomes less discouraging in its difficulty.

It has been asserted in the early part of this paper that, at very small intervals of stimulation, there was an increase in the height of the tetanic curve of two contractions which failed in fatigue, reappearing after rest.

We know that the elasticity of the muscle may vary greatly without change of form; a fatigued muscle during rest acquires new power of work.

<sup>1</sup> *Op. cit.*

<sup>2</sup> Marey, *Mouvement dans les fonctions de la vie*, p. 452.

<sup>3</sup> *Mechanische Leistung*, &c. p. 98.

<sup>4</sup> *Op. cit.*

Now if the first stimulus had the heightening effect on the muscle tension which we have supposed, there meets the second stimulus at its advent a sufficient new increase of elasticity to make its effect additive and distinct; whereas, with diminishing elasticity due to previous work, one can imagine how a quickly succeeding stimulus at the same interval as before should exert no cumulative effect upon the first contraction. The essential feature of the failure of a summation at a small interval of stimulation was that it occurred only after more or less prolonged double stimulation at that interval, over which the damping influence of the first stimulus might continue to such an extent as to make ineffective the second. In the work of Kronecker and Stirling, however, cited above, succeeding stimuli entered the muscle while it was undergoing continually changes in its physical properties due to the contraction from the first stimulus. No more satisfactory explanatory comparisons as to the rôle of elasticity in contraction can be made than those used by Marey<sup>1</sup> in illustration of his most ingenious theory of muscular contraction.

A tetanus differs functionally from a single contraction in the fact that in the second case the total shortening is aroused in a muscle excited in a single phase of elasticity, while in the first case the summation is the result of stimuli acting on the muscle in very different elastic conditions.

Now if it be true that stimulation itself excites changes in the muscle-substance during the latent period which shall alter the elastic properties of the tissue, there seems to be no reason why one cannot obtain a true tetanus from two stimuli succeeding each other at the smallest intervals; and theoretically the shortening from the first contraction would not necessarily precede that of the second, as Helmholtz found. According to Fick<sup>2</sup> the natural length of the muscle during contraction is a function of the time, regardless of extraneous conditions, and at any moment the tension is a definite function of the length at the instant.

Again, as the amount of the chemical processes in a given time rises and falls with the tension, we have the equation  $J=f(t, l)$ .  $J$ , the intensity of the chemical processes, says Fick, for a given muscle, probably increases with  $l$ , and at first increases and then diminishes with  $t$ . This conclusion seems strengthened by consideration of the results from contraction of artificially shortened muscle and inspection of Fig. 5. The above formula, however, takes no account of the variation of the tension (which is determined by the elastic properties of the muscle)

<sup>1</sup> *Mouvement dans les fonctions de la vie*, p. 456.

<sup>2</sup> *Muskelarbeit*, p. 67.



as due to its own alteration, which alone makes the equation of comparative significance. This last is the function known as the change in the irritability of the muscle, which I have endeavoured to connect with the elastic property of the tissue. Representing this increase or decrease of evolution of energy by  $E$ , and the force set free in a unit of time or intensity of chemical changes as above by  $J$ , we may assume provisionally that the value of the quantity  $E$  at any time depends upon the sum of the chemical transformations already undergone, and the interval  $T$ , supposed to be uniform, between the stimulations; i.e.  $E = f(\Sigma J, T)$ .  $E$ , then, represents the difference between the amounts of energy developed in the first and any other contraction of a series. In order to find out the amount of energy liberated in this last  $E$  should be added algebraically to the first  $J$  of a series with its proper sign.  $E$  probably increases with  $\Sigma J$  for a while with a plus sign, and then changes its sign with a further increase of  $\Sigma J$ ; and the rate at which the changes in  $E$  are brought about is the more rapid the smaller the interval  $T$ .

The general results arrived at may be collected as follows:

1. If a series of tetanic contraction curves, which arise from the succession of two simple contractions at various intervals, be laid off on the same base line, and the envelope of the tetanic curves be constructed with abscissa units which mark the intervals at which the two single stimuli succeeded each other to give the tetanic contractions of the heights measured on the corresponding ordinates; then the envelope curve will be irregular at the smallest intervals of stimulation, usually rising suddenly from zero. The curve then ascends gradually to a maximum, and afterwards falls somewhat more regularly and quickly, tending to become parallel to the base line at an interval between the single stimuli equal to the duration of a single contraction. The curves of two succeeding single contractions first fuse into a single unbroken curve when they follow each other at an interval of about .026 second, corresponding to 38.8 stimuli in one second. The maximum height of a tetanus from two contractions is obtained when these are added after an interval of about .048 second, corresponding to 20.8 stimuli in one second.

2. Every stimulation puts the muscle into a condition modifying its reaction towards succeeding stimuli. These variations need not be accompanied by a change of form. (Previous observers.)

3. Two succeeding maximal contractions may probably produce an increased tetanic contraction when following one another at an interval

To answer these questions Goldstein adduces five sets of experiments, which I will briefly recapitulate, since my work was undertaken in consequence of the fact that they seemed not to justify all the conclusions he had drawn from them.

Experiment I. The animal (dog) is placed in a box and heated, its nose being exposed; the frequency of the respirations increases as the temperature goes up. The animal is taken out when its temperature has reached  $41.2^{\circ}\text{C}$ . Affusions of cold water have no effect in reducing the rate of respirations, except momentarily.

Goldstein thinks that this experiment proves that the increased temperature did not act by way of the skin, because cold affusions did not at once destroy its effects. If, however, we look at the Table given by him<sup>1</sup> we see that there is a fall from 110 to 92 respirations per minute, where we read in the notes: "From time to time cold affusions," and I would further suggest that the heated blood bathing the cutaneous nerves directly is certainly as strong a factor as the cold water poured on the skin, covered as it is with hair. I think, therefore, that this experiment does prove satisfactorily what Goldstein claims for it, and still leaves it undecided whether the increase of the number of respirations is brought about by way of the nerves of the skin, or otherwise.

Experiment II. A cat which had been made apnoëic when at the normal temperature is placed in the apparatus, and after the temperature has risen from  $37.4^{\circ}$  to  $39.5^{\circ}$ , artificial respiration is again tried, but in vain, no apnoëa is produced; the respirations going on at the same frequency. This experiment agrees altogether with those of Ackermann, and, as I shall point out later on, with my own.

Experiment III. A dog, into the veins of which 0.06 grm. morphine was injected, was placed in the apparatus, as in Experiment I. The temperature of the animal rises from  $38^{\circ}$  to  $40^{\circ}$ , while the respirations go up from 16 to 366 per minute. This experiment was made to show that it is not the cerebrum, or any feeling of uneasiness, which produces the increase in the rate of respiration. There are, however, some figures in the Table<sup>2</sup> belonging to this experiment which call for notice here. Goldstein, as we shall see further on, comes to the conclusion that the stimulation of the nerves of the skin is not the first cause of the increased rate of respiration; but a closer examination

<sup>1</sup> *Op. cit.* p. 82.

<sup>2</sup> *Op. cit.* p. 84.

of this Table would seem to contradict such a conclusion. We see there that the temperature of the animal is falling before and after being in the apparatus. At 10 h. it is  $39.6^{\circ}$ , at 10.30 when the animal was placed in the apparatus it had fallen to  $38.8^{\circ}$ . At 10.42 it has fallen to  $38.6^{\circ}$ . When the animal was placed in the apparatus the respirations were 16 per minute; 12 minutes afterwards, while the temperature of the animal has been falling  $0.20$  degrees, the respirations have gone up from 16 to 44 per minute. This seems to make it probable that the warm air acting on the skin causes the increase in the respiratory rhythm.

Goldstein has indeed himself seen the difficulty; but gives the following explanation: "That in this case a distinct increase in the number of respirations can be observed with a bodily temperature of  $38.6^{\circ}$  and  $38.8^{\circ}$  may be explained by the circumstance, that the temperature of the body has risen considerably, before the thermometer in the vagina has indicated this." If Goldstein cannot trust the indications of his thermometer here, why should he trust the instrument on any other occasion?

Experiment IV. The vagi are cut, the animal is heated up, as before, the respirations per minute rise from 5 to 310, while the temperature of the animal changes from  $37.3^{\circ}$  to  $40.2^{\circ}$ : an experiment performed to show, and as I think, showing, that it is not the terminal pulmonary expansion of the vagus upon which the increased temperature acts primarily.

To show that the heated blood would and could influence the medullary centre directly without action upon the peripheral nerves, Goldstein devised the following experiment.

Experiment V. Two tubes 3.7 cm. long, 1.2 thick, provided with tubules at both ends, so that hot water could be passed through them, were employed. These tubes were provided with a groove running lengthwise deep enough to place the carotid of a dog into it comfortably. The artery could thus be exposed to an increased temperature by the heated water running through the tubes. Water of  $54^{\circ}$ ,  $59^{\circ}$ , and  $71^{\circ}$  was employed with the object of heating up the blood as it passed through the artery resting on the heated tube.

But can the blood really be heated by this method? Let us look at the figures. At 10.45 the animal respires 26 times per minute; water at  $54^{\circ}$  C. is made to pass through the tubes; at 10.47 the respirations have risen to 50 per minute. Again at 11.10 the respirations are

16; water of 59° passes through the tubes; the respirations have increased to 62 per minute. Now have we any good reasons to think that in the short time of one or two minutes the blood running rapidly through the carotids and not directly over the heated brass at all, could be appreciably heated? Certainly Goldstein should have brought forward evidence on this point.

I have repeated all the experiments of Goldstein, and have found with him and Ackermann, (1) that by increasing the temperature of the surrounding air one can increase the respirations and the temperature of the animal; (2) that in such a condition the animal cannot be made apnœic; (3) that cutting the pneumogastrics does not prevent the increase in the respiratory rhythm; (4) that opium does not prevent it either. I have also repeated Experiment V. and found, it is true, that passing hot water through the tubes carrying the carotids, as described above, would increase the number of respirations. But I found also that the same increase in the number of respirations took place, when the arteries were clamped so that no blood could pass through them to the medulla. The increase is really, as one cannot fail to observe, brought about by pain; for it must be remembered that water at 54°, to say nothing of 71°, is decidedly painful to the hand. That it was pain that called forth these rapid respirations is shown by the fact that when I let water of the same (54°) temperature run into wounds made in the thighs, the same increase in the respiratory rate occurred.

I give a few figures. The number of respirations without water running through the tubes was 17 per minute; water of 54° was allowed to run through, the respirations went up to 46 per minute. The flow of the water was stopped and the arteries clamped above, on the cranial side of the tubes. The respirations were then 17 per minute when no water was running through the tubes, and went up to 45 per minute when water of 54° was passed through the tubes. We see, therefore, the same increase, whether the blood in the heated arteries was allowed to run up into the cranium or not. Again in the same dog, after the pneumogastrics had been cut and some chloral given, the respirations went up from 5 to 16 only when the hot water was passed through the tubes around the carotids, and went up similarly from 6 to 12 per minute when water of the same temperature was allowed to flow into wounds made in the thighs of the animal. If further corroboration of the view that pain mainly caused the increased respirations be necessary, we might add, that in other animals when

well under the influence of opium the changes were less marked or altogether absent.

Goldstein's crucial experiment does not then prove, as he maintains, that the heated surrounding medium caused the increased respiratory rhythm by causing a rise in the blood-temperature, which hotter blood acting directly on the respiratory centre was the immediate cause of the increase; and the question remains still undecided in what way the changes in the respiratory-centre, causing the increased rate of breathing, are brought about when the animal is heated, by exposure to air of a temperature higher than its own.

I hope that by the following experiments, which are given in more detail, some light may be thrown upon this point.

The first experiment, Table I., which coincides with No. I. of Goldstein, is inserted mainly to have something to compare the others with, and for the sake of clearness and completeness.

As far as the method of observation and the apparatus is concerned both are very simple. The apparatus consists of a piece of sheet iron large enough to place the dog-board on it, and of a case or box of wood of corresponding size, open at both ends, with windows and other openings on the side. These and the ends are closed by cloths, which can be removed or lifted aside when necessary. A thermometer reaching down into the apparatus, through a cork, gives the temperature in the interior. The apparatus was readily heated by one or two gas flames, beneath the sheet-iron. The temperature of the animal was taken from a thermometer placed in anus or vagina and remaining there during the experiment. The respirations were counted with the eye, an assistant giving the time, generally for a whole minute, excepting when the respirations became frequent, when fractions of a minute only were taken. The very high numbers cannot be expected to be absolutely correct. (Table I.)

In this experiment the dog was placed in the apparatus in such a way that its mouth was within, so that it had to breathe a warmed atmosphere.

To take a correct view of these experiments one must remember that under circumstances in which a man perspires a dog's respirations increase in number. While we sweat to keep cool the dog pants to keep cool.

Upon an inspection of this table one cannot fail to be struck by the rapid and great increase in the respirations, with but a trifling increase in the temperature of the blood; *e.g.* increase in body-tempera-

Table I.

April 23, 1879. Male dog. Small dose of morphia.

Time.	Surrounding temp.	Respirations.	Temp. of animal.	
7-06	22	53	39.5	
7-08	53			Animal placed in apparatus.
7-14	48	66	39.7	
7-17			39.8	Can be heard panting.
7-22	43	180	39.8	
7-29	43	220	39.8	
7-40	51	280	40.3	
7-48	55	310	40.5	
7-59	55		40.8	Respiration cannot be counted.
8-00	12.5			Taken out of apparatus, on an exposed, cool place.
8-05	12.5	180	40.6	
8-13	12.5	166	40	
8-21	12.5	120	39.3	
8-26	12.5	57	38.6	
8-30	12.5	38	38	
8-39	12.5	24 (?)	37.8	
8-40	45			Placed back in warm apparatus.
8-42	45	26	37.8	
8-46	48	27	37.8	
8-50	48	36	37.9	
8-54	50	60	38	
9-00	54	132	38.4	Begins to pant.
9-13	46	200	39.2	
9-22	46	240	39.6	
9-23	12.5			Taken out on an exposed cool place.
9-29	12.5	180	39.4	
9-32	12.5	194	39	
9-39	12.5	112	38.5	
9-49	12.5	24	37.8	

ture 0.3° C.; increase in respirations from 53 to 180. This fact alone would make it very probable, that the nerves of the skin were the active agents in bringing about these changes.

That however the increased frequency in the respirations can be brought about by the heated air surrounding the skin and stimulating the nerves there, without heating the blood at all, can be gathered from Table II., and one can further see it exemplified by watching a dog on a warm summer-day.

Table II.

April 25, 1879. Bitch. No opiate.

Time.	Surr. temp.	Temp. of dog.	Resp.	
7-30	19	39	22	
7-38	19	38.9	22	
7-39				Animal placed in warm apparatus.
7-42	47	38.9	42	
7-45	48	38.9	66	
7-47	49	38.9	214	
7-54	50	38.9	180	
8-09	51	38.9	320	
8-30	43	38.9	340	
8-45	49	38.9	300	Animal taken out and placed on an exposed cool place.
8-50	19	38.9	268	
8-52	19	38.9	220	
8-59	19	38.9	140	
9-06	19	38.9	120	
9-11	19	38.9	50	
9-14	19	38.9	28	
9-18	19	38.9	24	

We see here that the animal being loosely tied and having its nose not far from a large opening in the apparatus so that it could inhale the cooler air of the room, by panting vigorously, or otherwise, managed to keep its blood cool. The body-temperature was not elevated at all, while the respirations were increased from 16 to over 300 per minute. The enormous frequency of respiration prevented its blood from becoming warmer, and clearly had some cause apart from heated blood as its stimulus.

To investigate the influence which the nerves of the skin have in this, the spinal cord was divided at the bottom of the neck in another dog, and the animal placed in the warm box with its head and neck protruding. By this means afferent nervous impulses from the heated skin were to a very large extent cut off, but the raising of the temperature of the blood was not interfered with.

This experiment, Table III. (since by section of the cord the nerves of the skin that were heated could not act on the medulla, and since those of the head and neck were not exposed to the heat excepting by way of the heated blood circulating in those parts), shows that the blood itself directly, without intervention of the skin-nerves (at least the

Table III.

May 5th, 1879. Young dog. Cord cut about 4.30. Placed in apparatus in such a way, that the paralyzed and anaesthetized parts only were subject to heat directly. The head was free, so that the dog could inhale air at the temperature of the room. Post mortem examination showed the cord to be cut between the fifth and sixth cervical vertebrae.

Time.	Surr. temp.	Temp. of dog.	Resp.	
5-07	26	37.5	15	Apparatus is heated up.
5-12	42	37.4	15	
5-20	51	37.6	14	
5-29	56	37.8	15	
5-35	59	38.3	18	
5-40	60	38.8	20	
5-49	62	39.5	29	
5-54	58	40.0	31	
6-00	57	40.8	31	
6-07	56	41.5	79	
6-08				Taken out of apparatus.
6-11	23	41.6	64	
6-17	23	41	41	
6-26	23	40.4	38	
6-30	23	39.9	28	
6-38	23	39.5	26	
6-46	23	39	24	
6-53	23	38.5	22	
6-58	23	38.1	20	
7-06	23	37.6	18	
7-14	23	37.1	18	

great bulk of them), can raise somewhat the number of respirations. But there is a difference between the two modes, as can be seen by comparing Tables I. and II. on the one hand with Table III. on the other. In I. and II. where the skin is heated, either alone as in II. or together with the blood, we see an increase of the respirations from 53 to 310 respirations with an elevation of temperature from 39.5 to 40.8, and in No. II. from 22 to 340 respirations, where we have no increase in the temperature of the dog's blood, while in No. III. the highest figure for the respirations is 79 (the animal beginning with 15), with a change in the temperature of the animal of 3 degrees. There was noticed also a difference in the character of the respirations in the two kinds of experiments. While



in I. and II. the animal soon showed that complex of processes known as panting, this did not occur in III. It is of course possible that this might have set in if the temperature had been raised still higher.

An interesting experiment is the following Table IV. which not only supports No. III. but may help to solve another question.

Table IV.

May 20, 1879. Bitch. Tracheotomized and lower cervical cord cut. When in apparatus, neck and head free and exposed.

Time.	Surr. temp.	Temp. of dog.	Respir.	
7-30				Trachea and cord are cut.
7-39		38.8		Artificial respiration for 2 minutes, producing apnœa lasting 1 minute.
7-51		38.2	32	
7-53	44			Animal placed in apparatus.
8-03	49	37.8	27	
8-09	50	38.2	27	
8-12	51	38.4	32	
8-20	48	39.1	46	
8-23	50	39.5	48	
8-29	50	40.1	56	
8-33	50	40.6	130	
8-36				While the animal is still in appa- ratus, artificial respiration is kept up for 2 minutes, producing apnœa of about 1 minute.
8-40	49	41	100	
8-41				Animal taken out on an exposed cool place.
8-45	21	41.3		Artificial respiration for 2 minutes, producing apnœa of about $\frac{3}{4}$ min.
8-48	21	41	84	
8-52	21	40.4	60	
8-55	21	40		Artificial respiration of 2 minutes, apnœa of 1 minute.
8-58	21	38.6	36	
9-04	21	38	40	
9-10	21	37.6	36	

It will be remembered that Ackermann as well as Goldstein and myself did not succeed in producing apnœa in an uninjured heated animal. But when the cord is cut matters are different. Three times without failing in any trial did I succeed in the same animal in bringing about apnœa, once while the animal was yet in the apparatus

and while its temperature was on the increase, and twice after the animal had been taken out of the box. The figures show that this was not due to a diminution of the temperature of the dog itself, for this was even rising while the respirations were in abeyance after artificial respirations. The last two experiments then show that the respiratory centre can be influenced (in these cases stimulated) by changes in the blood brought about by heating the animal.

There arises now however another question which we think is partly answered by these experiments; that is: How does the blood act when it increases the number of respirations, does it stimulate the centres by virtue of its increased temperature, or does it act by virtue of the increased vascosity (to use a short phrase)? For we may assume that an increase of the temperature of the tissues will increase also the chemical changes in them.

The last experiment would seem to point in the direction of the chemical changes as the effective causes, and that the increased temperature—at least within certain limits—has none or only a slight direct effect on the respiratory centre. For although the temperature of the blood remained high (40° to 41°), artificial respiration, which charged the blood with oxygen but, as shown by the thermometer, did not reduce its temperature, produced apnoea lasting three-quarters of a minute, while when the animal had been at the normal temperature the same amount of artificial respiration produced apnoea lasting a whole minute; this difference being probably due to the increasing changes proceeding in the body using up the oxygen at a more rapid rate than under normal conditions.

This leads us to consider some statements made by Ackermann in the October number, 1866, of the *Deutsche Archiv für Klinische Medicin*. Ackermann there publishes the results of investigations on animals which he had exposed to increased temperatures in various ways.

One of the conclusions at which he arrives is that not only the skin but also the lungs are used as an apparatus to regulate the body-temperature; the skin acting in a more gross manner, the respiratory mechanism being used in bringing about the minor changes and adaptations.

This sentence my experiments fully confirm, especially that showing the increase of respirations due to exposing the skin of the animal to an elevated temperature, while that of its blood was not affected.

Ackermann makes however another statement (p. 361). He says:

"This increase in the frequency of the respirations has its cause not, as might be expected, in a want of oxygen in the blood or in an excess of carbonic acid but alone in the increase of the temperature of the whole organism. We must recognize a heat-dyspnœa.....At high temperatures of the animal, artificial respirations have no influence on the frequency of its respirations, not even when in consequence of the inflations the blood shows a bright red colour in the veins, while by artificial respirations at a lower temperature the number can be greatly reduced, and at ordinary temperatures even brought to a standstill."

For temperatures not exceeding certain limits, the experiments communicated in this paper would seem to clear up this matter. The attempts to produce apnœa may prove unsuccessful because the respiratory centres, though not stimulated by the venous character of the blood, yet are constantly influenced by powerful peripheral stimulation of the heated final distributions of all the ordinary sensory nerves of the skin and other tissues. When the nerves, or more accurately a large portion of them, were excluded by section of the cord apnœa could be produced, though it seemed not to last quite as long as at normal temperatures.

Conclusions :

1. Goldstein's experiment with the tubes is inconclusive.
2. The increased respiration following exposure of the animal is due to two causes, skin-stimulation and warmed blood.
3. Of these, skin-stimulation is the more powerful.
4. Apnœa can be produced in heated animals if skin stimuli be cut off.
5. The direct action on the respiratory centres of the hotter blood of the heated animal is probably not, or not only, due to its temperature but to its greater venosity.

As regards the influence of the heat of the blood, *per se*, I add this restricting word "probably," because though 40° or 41° may not have acted as direct stimuli in the cases given, higher temperatures in these animals, and these same in other animals, may act differently from lower temperatures. I hope that I may take up this work in the future and discuss the effects of higher temperatures.

In concluding, I express my thanks to Prof. Martin, who not only suggested this interesting topic, but also directed the work and helped in various ways in its execution.

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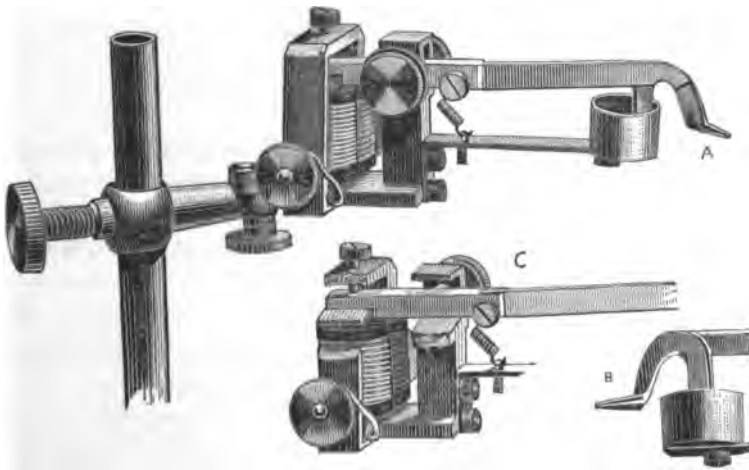
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## A SELF-FEEDING CHRONOGRAPH PEN.

By H. NEWELL MARTIN, D.Sc., M.A., M.B., *Professor of  
Biology in the Johns Hopkins University.*

THE little instrument here described presents no novelty of idea, but has proved so handy and useful in the laboratory of the Johns Hopkins University, that I have thought well to describe it for the benefit of others. The idea was suggested to me by a similar instrument used by my colleague Prof. Rowland in the physical laboratory for writing on a horizontal surface, which, with a little trouble, I succeeded in modifying so as to fit it to write on the vertical paper of Ludwig's kymographion. Whoever has worked much with that instrument has probably been frequently annoyed by having his chronograph or stimulation pen give out at some critical moment, since the glass pens commonly used do not hold enough ink to last throughout a prolonged experiment. Moreover, when the instrument is not running, they are very apt to trickle down the paper. The self-feeding pen, once charged, will last for hours without fail and does not trickle.



The instrument is represented, three-fourths its actual size, in the woodcut, A. Its essential part, consists of a brass lamina

curved in a vertical plane for most of its extent, and having a deep but narrow groove cut in its convex edge. One end dips into the little brass cup, represented along with the brass lamina alone at B; this, when the pen is in use, is filled with the ordinary kymograph ink. The other end tapers off to a bluntly rounded writing point and the pen is bent so that this part lies at right angles to the rest and in a horizontal plane. In the horizontal portion the groove is carried through so as to form a complete slit with a "nib" above and below it. If the other end of the pen be dipped in the brass cup the writing point will not always be charged by capillarity, but if the groove be once filled by dropping a little ink from a fine pipette into its highest part, and this ink be spread out if necessary between the nibs, by passing a strip of paper between them, the pen will thenceforth keep itself filled from the reservoir, so long as there is any ink in the latter.

The accessory parts of the instrument are easily understood from the figures and may, of course, be greatly modified according to the end in view in different cases. The pen proper, in the form I find most convenient, is carried on the long arm of a lever, which is depressed by a little brass spring. The short arm of the lever carries a soft iron armature which is attracted by an electromagnet whenever the circuit in the latter is closed, whether by a seconds clock or otherwise. The pen point is thus raised and remains so until the circuit is broken, when the spring above mentioned again depresses it. The screw shown above the armature regulates the amount of these excursions; it should in all cases be so fixed that the end of the pen does not strike against the bottom of the reservoir; otherwise the ink in the latter splashes. The whole instrument is carried on a short horizontal arm movable up and down a vertical support. Where the carrying arm joins the instrument proper is a joint permitting free motion in a horizontal plane. The vertical rod being in addition movable, by means of a slot in the plate which carries it, in any horizontal direction for more than two inches, the position of the writing point is easily adjusted. It should be arranged so that the axis of the horizontal part of the instrument is nearly perpendicular to the paper, and so that only the exact tip of the writing point touches. That half of the lever next the pen, being made of thin brass, acts as a spring by which the pressure of the pen point on the paper can be regulated.

I commonly now use two such pens with the kymograph; one for the "time," the other for the "stimulation" record. They are both carried on the same vertical rod and the upper one is modified so that its electromagnet is above the lever instead of below it. The lower instrument can then be brought close up, so that the writing points of the two come within an inch of one another on the same vertical line. By slight modifications, removing the upper reservoir out of the way of the lower pen, the two could readily be brought still closer together if desirable; but as my kymographs are Fulcher's new ones with an eight inch wide roll of paper I have never been cramped for room.\*

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\*The instrument may be obtained from T. Schneider, Mechanician, Johns Hopkins University, Baltimore, U. S. A., or from B. Fulcher, Pantons Street, Cambridge, England.





**OBSERVATIONS UPON THE EARLY STAGES IN  
THE DEVELOPMENT OF THE FRESH-WATER  
PULMONATES.** By W. K. BROOKS, PH.D., *Assistant Pro-  
fessor of Comparative Anatomy in the Johns Hopkins University.*

IN the present state of our knowledge we must regard as an unsolved problem and a fair subject for observation and discussion, the interesting and fundamental morphological question whether the germ layers of the various Metazoa are to be regarded as homologous structures which owe their resemblances to descent from an ancestral gastraea.

When we reflect upon the striking resemblances between the early stages of the most widely separated Metazoa, resemblances which do not seem to admit of a mechanical explanation, it is hard to resist the growing conviction that when the evidence is all in it will establish some phylogenetic hypothesis to explain them. At the same time we cannot overlook the serious objections which have been made by various writers to the acceptance of any of the modifications of the gastrula theory which have been proposed.

Before we shall be in a position to form an opinion upon the whole subject a number of subordinate questions must be settled. One of these is the question whether the early stages in the development of the endoderm, the digestive cavity, the mouth and the anus are enough alike in various Molluscs to be reconciled with any such theory.

If we assume the accuracy of all the papers upon the development of Molluscs which have been published since this question began to attract attention we must conclude that there is no such similarity, since it is almost impossible to find two accounts of the process which are at all alike.

A few references will show this very clearly. According to Rabl (*Ueber die Entwicklungsgeschichte der Mahlermuschel*, *Jenaische Zeitschr.*, 1876), the macromeres of the segmenting egg give rise to the endoderm and are carried into the body cavity by a process of invagination, which results in the formation of a gastrula. The blastopore, which is situated upon the dorsal surface of the body, soon closes up, and its place is occupied by the shell, while the

endoderm leaves its primitive position and travels to the ventral surface of the body-cavity, where it unites with the ectoderm and forms a new external opening.

Salensky says (*Bemerkungen über Haeckel's Gastraea Theorie*, Arch. f. Naturges., 1874, p. 168, Taf. V, Figs. 1, 2 and 3), that in the Oyster there is nothing like a gastrula stage, and the endoderm is a solid central mass of cells at a time when the velum, shell, and mouth invagination are present, while the digestive cavity and its wall are of later formation.

Ray Lankester (on the Coincidence of the Blastopore and Anus in *Paludina vivipara*, Quart. Journ. Mic. Sc., Oct., 1876), and Bütschli (*Zur Entwicklungsgeschichte von Paludina vivipara*, Zeit. f. Wiss. Zool., July, 1877), agree that in *Paludina vivipara* there is a gastrula stage, and that the primitive digestive cavity becomes the permanent cavity, while the blastopore occupies the position of the anus, and the mouth is formed later by an invagination of the ectoderm to meet the digestive tract.

Fol has studied the development of a number of Pteropods and Heteropods (*Études sur le Développement des Pteropodes*, Arch. d. Zool. exp. et gen., 1875, 4. 1), and holds that in these molluscs the blastopore becomes the mouth.

Brobetsky reaches a nearly similar conclusion (*Studien über die embryonale entwicklung der Gasteropoden*, Arch. f. Mic. Anat., 1876, XIII), and says that in a number of Prosobranchs studied by him the mouth is formed at the point where the ectoderm closes in over the food-yolk.

Salensky says (*Beiträge zur entwicklungsgeschichte der Prosobranchien* Zeit. f. Wiss. Zool., XXII, 1872, 4, 28-454), that in certain Prosobranchs the mouth and oesophagus arise from an invagination of the ectoderm, and the rest of the digestive tract from the endoderm; and Kowalevsky says (*Sitzungsberichte der Zool., Abtheilung der III Versammlung russischer Naturforscher* (Zeit. f. Wiss. Zool., XXII, 1872, p. 283), that in *Vermetus* and another Prosobranch which he has studied the invagination which, according to Salensky gives rise to only the mouth and oesophagus, really forms the whole digestive tract.

The above references which might be greatly extended, show the impossibility of any general conclusion in the present state of our knowledge, for the statements of different observers can no more be reconciled with any of the modifications of the gastrula

theory which have been proposed by Salensky, (loc. cit.) Von Jhering, (Ueber die Ontogenie von *Cyclas* und die Homologie der Keimblätter bei den Mollusken, Zeit. f. Wiss. Zool., XXVI, 414), and Lankester (Notes on Embryology and Classification), to account for the phenomena of molluscan development, than with the original theory, and almost the only general conclusion which can be drawn from them is that we need new observations and increased information.

I have been engaged for some years in studying the early stages of Molluscs, and have directed especial attention to the history of the digestive tract. Though I am not yet prepared to undertake a general discussion of the subject, I have traced the digestive tract in a number of forms, and am therefore able to give purely descriptive information, which will serve as a basis for future discussion. The present paper is an account of observations upon the fresh-water pulmonates, for although there are numerous published accounts of the embryology of this group of Molluscs, the statements and opinions in regard to the digestive tract are fully as contradictory as those which we have quoted.

The two most valuable and generally accurate papers on Pulmonate development, that by Lankester, (Observations on the development of the Pond Snail (*Lymnaeus stagnalis*), and on the early stages of other Mollusca. By E. Ray Lankester, M.A., Fellow and Lecturer of Exeter College, Oxford. Quart. Journ. Mic. Science, N. S., XIV., p. 365-391; Plates XVI and XVII.) and that by Rabl, (Die Ontogenie der Süsswasser-Pulmonaten, von Carl Rabl, Jenaische Zeitschr, IX, 2 May, 1875, p. 195-240, Taf. VII-IX.) leave this particular point in Pulmonate development in great obscurity. Lankester's figures point to the conclusion which I have reached that the invagination neck gives rise to the rectum, but moves from its primitive position to open by a definitive anus which has no connection with the blastopore, while the mouth is an independent invagination of the ectoderm. The author confesses (pp. 376, 383 and 385) that his observations of those early stages which alone could give definite information upon this point, are incomplete and unsatisfactory, and in later papers ("On the Coincidence of the Blastopore and Anus in *Paludina vivipara*," and "Notes on Embryology and Classification.") he says that in *Limnaeus* both the oral and anal outgrowths make their appearance upon the site of the elongated blastopore; a

conclusion which appears to be as greatly at variance with his own figures as it is opposed to my observations.

Rabl was unable to satisfactorily decide what relation the blastopore bears to the definitive mouth and anus, and says that although he was not able to trace the transformation of the gastrula opening into the anus he thinks such a transformation more probable than its change into the definitive mouth.

Rabl's and Lankester's accounts agree in describing a gastrula stage in which there is a layer of endoderm surrounding a digestive cavity with an external opening, the blastopore.

According to Ganin and Von Jhering there is nothing like a gastrula stage, and their accounts are quite different from those of the two observers above quoted. Ganin's paper (*Beitrag zur Lehre von den embryonalen Blätter bei den Mollusken*, Warschauer Universitätsberichte, 1873, I.) I know only from the abstract by Hoyer in Hoffmann w. Schwalbe's *Jahresberichte*, (1872, I, p. 35.)

I judge from this abstract that he believes the endoderm of Rabl and Lankester to be a food yolk, and the ectoderm of these writers, a blastoderm, which entirely covers up the food yolk, and becomes divided at one point into an ectoderm, mesoderm and endoderm; the latter layer becomes invaginated to form the digestive tract, which is between the ectoderm and the food yolk, and which opens externally by an aperture which becomes the definitive mouth, while the anus is formed later at the opposite end of the body.

Von Jhering's view is given at some length in his papers upon the development of *Helix* and of *Cyclas*, ("Ueber die Entwickelungsgeschichte von *Helix*. Zugleich ein Beitrag zur verg. Anat. und Phylogenie der Pulmonaten," von Dr. Hermann von Jhering in Göttingen. *Jenaische Zeitschr*, 1875, IX, III, 299-377, Taf. XVII-XVIII, and "Ueber die Ontogenie von *Cyclas*, und die Homologie die Keimblätter bei den Mollusken." *Zeit. f. Wiss. Zool.*, XXVI, p. 414-433.)

By a most ingenious method of interpretation he succeeds in showing that all the published accounts go to prove that there is one type of development common to the Pulmonates, Nudibranchs, Tectibranchs, Pteropods and Cephalopods, or all the representatives of his Phylum *Platycochlida*.

In all of these, he says, the primary endoderm, which never forms a primitive digestive cavity, is in part or entirely resorbed, and the digestive cavity with all its appendages is formed from the ectoderm.

These abstracts show the great lack of agreement among the writers upon the subject, and are a sufficient reason for the publication of new observations upon the development of Pulmonates.

I have been able to obtain a very perfect series of stages in the development of *Physa*, *Limnaeus* and *Planorbis*, and trust that the observations which are detailed in this paper will be regarded as final for this group, and will thus contribute the beginning of a basis for the general discussion.

As the origin of the digestive tract was made the main object of my investigations, I have used every effort to actually see the living egg pass through all the stages of development. To do this requires such close and constant attention that it leaves no time for the more minute study of eggs which have been treated with re-agents, and I have no observations to offer upon the fascinating subject which is now receiving so much attention from embryologists; the history of the changes which result from fertilization, and the origin and behaviour of the segmentation nuclei.

On the other hand the actual observation of the change of the living egg from one stage to another, brings into prominence certain features which would be entirely lost sight of in the study of a series of perceived specimens.

An interesting fact, which is very conspicuously shown when the segmenting eggs of the fresh-water Pulmonates are kept under continuous observation, is the rythmical nature of the process of segmentation. Various observers have noticed that the segmentation of the egg is not always a continuous process, but that it is interrupted in certain animals by intervals of inactivity, and Flemming has given figures and brief descriptions of the egg of *Anodonta*, during two of these periods of rest. (*Studien in der Entwicklungsgeschichte der Najaden. Sitzb. der K. Akad. der Wissensch*, Feb. 1875, von Walther Flemming, pp. 44 and 45, Figures 6 and 10, Taf. II.)

As the following description will show, the segmentation of the Pulmonate egg is divided into extremely well marked alternating periods of activity and of repose.

When a period of activity has commenced the changes follow each other quite rapidly, and the spherules which are formed by the process of division stand out sharply and prominently, but as soon as the period of activity is succeeded by a period of rest the spherules lose their distinctness, and the egg gradually assumes a more or less perfectly spherical form.

During the period of segmentation the protoplasm of the whole egg gradually becomes more and more transparent, on account of the gradual disappearance of the granular matter which it contains, and the rythmical nature of the process of segmentation would seem to admit of a simple explanation on the hypothesis that the elasticity of the protoplasm offers a resistance which must be overcome before the force which is set free by the assimilation of the granular food material can exert itself to produce the active changes of segmentation. During a period of rest the process of digestion accumulates a supply of energy which at length becomes sufficient to initiate a period of activity, which continues until the whole of this reserve has been expended in the rearrangement of the protoplasm. The physical properties of the protoplasm now reassert themselves, and tend to reduce the whole mass to a spherical form again, and the egg remains inactive until the supply of energy again becomes great enough to overcome this resistance.

The eggs of the various genera of fresh-water pulmonates are very much alike although the egg-clusters present considerable variety. It is true that the eggs themselves have distinctive characteristics such as size, transparency &c., but these differences are very slight.

The mode of development, is not precisely alike in the various species, but the resemblance is so great that figures of the early stages of *Physa* answer for a description of the development of *Lymnaeus* or *Planorbis*.

The species which I have studied most carefully are *Physa heterostropha* and *Planorbis parvus*, and some of the drawings are from one, some from the other. Most of the stages figured were observed in both, but the resemblance is so close that it did not seem necessary to duplicate drawings of stages which had already been figured.

At the time of extrusion, Figure 1, the germinative vesicle of the egg is still visible near the centre of the yolk, although impregnation takes place before the eggs are laid. The yolk is spherical; uniformly granular, and there are no indications of an investing membrane. The opaque matter of the yolk of the egg of *Physa*, Figure 1, is in the form of minute granules, but in *Planorbis*, there are, in addition to the granules, numbers of larger spherical globules of deutero-plasm, most of which are situated near the surface of the egg. These are shown in Figure 7 which is a later stage of the egg of *Planorbis*.

A few minutes after the egg is laid the germinative vesicle becomes invisible, the yolk shrinks a little and is now seen to be surrounded by a delicate vitelline membrane, Figure 2, between which and the surface of the protoplasm of the yolk is a transparent layer, *b*, apparently occupied by a watery fluid. At one point this space is enlarged so as to form a lens-shaped cavity, *c*, into which the polar globules are soon discharged.

In a few minutes the egg, Figure 3, elongates along an axis perpendicular to the point where the polar globule is making its appearance; the transparent layer disappears, and the yolk is again pushed out into contact with its membrane except around the polar globule. At this point, the formative pole of the egg, the membrane may still be seen, roofing over a broad shallow furrow, the commencement of the first cleavage plane. In from five to ten minutes this furrow becomes a deep fissure, Figure 4; a second polar globule makes its appearance and the first pushes its way through the vitelline membrane. At the same time a second furrow pushes its way in at the nutritive pole, and in about five minutes more, Figure 5, this second furrow has pushed into the yolk, towards that which first made its appearance at the formative pole of the egg, and the yolk is now divided into two nearly spherical portions united by a narrow neck. The second furrow is however only about half as deep as the first, the vitelline membrane does not stretch across it. In fifteen or twenty minutes after oviposition the two primary segments become completely separated from each other as in Figure 6, and each of them now has a clearly defined spherical outline. With the completion of this division the first period of segmenting activity ends, and is quickly followed by a period of rest, during which the results of the first period of activity are gradually obliterated. Two or three minutes later than the stage shown in Figure 6, the egg from which this figure was drawn had become nearly similar in outline to Figure 3, except that the furrow at the formative pole of Figure 3 was wanting in the later stage, and the polar globules were perched upon the surface of the yolk, and no vitelline membrane was visible.

Figure 7 is an outline of an egg of *Planorbis* at this stage, or five minutes after the completion of the first division.

In a surface view a transparent area can be seen through the yolk, and upon the axis of the first division, or principal axis.

Deeper focusing shows that the transparency is due to the presence of a lens-shaped segmentation cavity, which is enclosed peripherally by the union of the two primary segments. This cavity persists from this stage until the completion of segmentation.

In *Physa*, during the first period of rest, the egg becomes more nearly spherical than Figure 7, and, while the two primary spherules are in this condition, a second plane of cleavage appears at right angles to the first, and passing through the principal axis of the egg, divides it into two elongated segments, each of which is made up of one-half of each of the primary segments. In *Planorbis* this second division is somewhat different. At the beginning of the second period of activity the two primary segments again become sharply defined, and almost as distinct from each other as at the stage shown in Figure 6. Each of them then divides into two, and the four soon become spherical as shown in Figure 8, which is the formative pole of a *Planorbis* egg, at the end of the second period of activity. The four spherules are not similarly placed with reference to the principal axis, which is marked by the polar globules; but are arranged in pairs. The one at the right and the one at the bottom are the derivatives of one of the primary spherules, and the one at the top and the one at the left the derivatives of the other.

As soon as this stage is reached the four spherules begin to contract and to approach each other and the egg passes into the second resting stage. Figure 9 is a view of the nutritive pole of the same egg, about four minutes after the stage shown in Figure 8. The fusion is not so complete as it was during the first period of rest, and the outlines of the spherules can still be made out in a surface view: their adjacent edges being united by a bridge of transparent very slightly granular protoplasm.

Deeper focusing shows that the centre of the egg is occupied by a quadrangular segmentation cavity, Figure 10, with its angles at the lines of union of the four spherules.

The end of the second period of rest is of especial interest, since we find that the segregation of the protoplasm of the endoderm and ectoderm now takes place, and is plainly shown before the commencement of the next period of activity which is to result in the separation of the macromeres from the micromeres. In a side view of the egg at the end of the second period of rest, Figure 11, each spherule is divided into three pretty well marked regions,



distinguished from each other by the amount of deuteroplasm contained in the protoplasm.

The formation end of each spherule is quite transparent, and contains none of the large spherules of deuteroplasm, and only a few granules. The central portion of each spherule is quite granular and contains many of the food spherules, but it is much more transparent than the yolk of the unsegmented egg. A large nucleus is also very conspicuous in this region.

Almost all of the food material is massed in an opaque ball at the nutritive pole of each spherule. A plane of division, perpendicular to the principal axis, now separates the transparent ends of the four secondary spherules, thus forming four small transparent micromeres, at the formative pole, and four much larger, more opaque macromeres at the nutritive pole; each of the latter having a mass of food material at its nutritive end.

The eight segmentation spherules quickly become distinct from each other and spherical, as in Figure 12, and the egg then passes into the third resting stage, and in about three minutes it has the form shown in Figure 13. From this time until the completion of segmentation the changes take place more rapidly, and the series of figures from 11 to 17 were drawn from a single egg; the process was traced and sketched in a great number of eggs however, and it may be followed in this species, *Planorbis parvus* without difficulty, as the egg cases are so small and contain so few eggs that they can be examined without pressure, even with high powers.

As the egg passes into the third resting stage the spherules become flattened and drawn together, the segmentation cavity becomes conspicuous, and the egg assumes the form shown in Figure 12, and a little later it becomes almost spherical as shown in Figure 13. In a surface view the outlines of the spherules can still be traced and they are united by transparent protoplasm, as in Figure 8. In an optical section, Figure 13, the lines of union are quite distinct, and the large drops of deuteroplasm shown in Figure 12, are seen to be restricted to the external surfaces of the macromeres. The third period of rest is followed by the fourth period of activity, during which the four micromeres remain passive and retain the shape which they had during the period of rest. The macromeres become spherical as in Figure 14, and the granular matter separates into two masses which are at first joined by a bridge of transparent protoplasm, but fission along this line soon

separates each macromere into two portions which then becomes spherical and well defined, as in Figure 15.

The spherule nearest the formative pole is much smaller and more transparent than the other, and is a micromere like the first set.

The four macromeres, together with the four last formed micromeres, now become flattened and pass into the resting stage, as shown in Figure 16, but before the flattening has reached its maximum, the four older micromeres swell up, become active, and divide. A surface view of the egg at the end of this, the fifth, period of activity, is given in Figure 17. The egg is made up of sixteen spherules, which are polygonal from mutual pressure, and are joined to each other by bands of non-granular protoplasm. At the formative pole are the polar globules, *po*, and the four oldest micromeres; below these are the four which were separated from the first four by the last or fifth segmentation. Wedged between these are the four macromeres with the masses of food material at their lower ends. The egg is now so thoroughly divided that it is difficult to trace the history of each spherule, but the appearances which were observed seem to indicate that segmentation now alternates between the two poles of the egg; the nutritive pole swelling up and dividing and then passing into the resting condition while active changes take place at the formative end.

Figure 18 is a surface view of an egg divided into thirty-two spherules, and undergoing segmentation at the nutritive pole.

The micromeres have now assumed the form of a definite layer of cells, the ectoderm arching over the segmentation cavity at the formative pole.

Figure 20 is a side view and Figure 21 a polar view of the next stage which was observed. The embryo now consists of four large opaque spheres, surrounded by a single layer of cells. At the stage figured, the polar globules have disappeared, and six eggs were found between those shown in Figures 19 and 20, but a comparison of the latter figure with those before it seems to show, beyond the possibility of doubt, that the top of this figure is the point where the polar globule was placed during segmentation, and that the four large spherules are the four macromeres which have become covered by a layer of cells, the product of the micromeres, spreading on all sides, from the formative towards the nutritive pole. Ray Lankester figures three views of an egg in about

the same stage as the one shown in Figure 8 of the present paper, (On the Development of the Pond Snail, and the Early Stages of other Mollusca, Pl. XVI, Figures 4, 5 and 6), but with the polar globule situated at the pole around which the four macromeres are arranged.

His next stage (Figures 7, 8 and 9), is a little older than our Figure 20, but the polar globule is where we should expect to find it; that is, at the point which is uppermost in our Figure 20. As the author more than half suspects the first stage, Figures 5 and 6, was incorrectly observed, and if he had given to the subject the "little more time and care" which he says would settle the point, he might have spared the long discussion of the relation between the early stages of *Lymnaeus* and those of *Aplysia* (pp. 375-6). The present series of observations shows conclusively that the Fresh-water Pulmonates resemble *Aplysia* and other invertebrates in having the formative pole of the egg indicated by the polar globule.

When the embryo at this stage is viewed from the point opposite the formative pole, Figure 21, a square area over the space between the four macromeres is seen to be still uncovered by the ectoderm. In the same view of a slightly older embryo, Figure 23, the shape of the embryo has undergone considerable change, and is now rectangular instead of square, with the short sides only half as long as the long ones. In a side view, Figure 22, the outline is more like that of the preceding stage, but the ectoderm cells are very much smaller at the formative pole than around the orifice of invagination. Internally, important changes have taken place. The four macromeres have become fused into a flat mass, which is bisquit-shaped in a side view, nearly circular when seen from above or below, and with no traces or division into spherules. The mass thus formed is much smaller than the four macromeres of the previous stage, and is separated from the ectoderm by a wide space on all sides except that where the orifice of invagination is placed. This opening or uncovered space is now rhomboidal with its long axis transverse to the longest axis of the embryo.

Deeper focusing did not show any cavity beneath it, and the opaque mass appears to be solid. The fusion of the macromeres must not, however, be mistaken for the obliteration of a primitive digestive cavity. No such cavity has so far been present, and I take it that the fusion of the macromeres is simply a change simi-

lar to those described earlier where a period of activity was shown to be preceded by a period of rest, during which previously acquired characteristics became obscured.

As I understand the present stage, the four macromeres have passed into a period of rest, which is to be followed by the division of the endoderm into cells, and the formation of a digestive cavity. The stages shown in Figures 20, 21, 22 and 23 must be of very short duration, for only three egg-cases were found with embryos in this condition, and they seem to have escaped the notice of all other observers except Ray Lankester, whose Figures, 7, 8 and 9, already noticed, represent about the same stage of development.

In his paper upon the "*Ontogenie der Süßwasser Pulmonaten*" (*Jenaische Zeitschr.*, IX, 2, 1875), Rabl describes and gives several figures of the segmentation and the formation of the gastrula, but his account of the process is quite different from that here given. He has entirely overlooked the present stages, and his Figures 8 to 11, Plate VII, are entirely imaginary. Figures 12 and 13 are drawn from embryos which were very much farther advanced than he supposed, and which would furnish no information as to the manner in which the primitive digestive cavity is formed.

The next stage of development, Figures 24, 25 and 26, was met with very frequently.

The embryo is now nearly spherical in general outline, although no profile view is perfectly circular. The ectoderm cells are quite small and polygonal, and the body is covered with small cilia which keep it in constant rotation. The various profile views which are presented, as the embryo spins around, are so much alike, and are seen for so short a time, that the examination of this stage is very confusing. If a number of outline sketches are made, it will be found that there are only two symmetrical aspects, and both of these are irregularly hexagonal. In one of the hexagonal faces, Figure 24, there is a lozenge-shaped opening, with one axis much longer than the other. This opening is undoubtedly the same as the opening, or uncovered space in Figure 23. In a surface view the centre of the body is seen to be occupied by an opaque ring, concentric with the opening, and surrounding a more transparent area which lies directly below the opening. Deeper focusing, Figure 25, shows that the transparent area is a single layer of endoderm cells around a small central cavity which is

continuous with the external opening. Outside the layer of cells is an opaque mass which is very granular and shows no trace of a division into cells, or at least none which can be made out through the body-wall. At one point *b*, this mass is separated from the ectoderm by a transparent space which seems to be part of the original segmentation cavity. The rest of the surface of the opaque mass, as seen in this view, is connected with the ectoderm by a transparent layer, *m*, which seems to be the beginning of the mesoderm. The cavity, *b*, is much more conspicuous at some times than at others, and may be seen to slowly expand as if it were being distended by a fluid, and then to quickly contract. A comparison of this stage with the previous one indicates that the external portion of the opaque mass of Figure 20 has separated from the remainder, to form the endoderm, which has been pushed inwards to form the digestive cavity, which opens externally through an orifice which may be regarded as the gastrula mouth. This process results in the transfer of the remainder of the opaque mass into the space between the ectoderm and endoderm, where it forms the opaque ring.

According to this view the endoderm is formed by a very slight modification of the process which gave rise to the ectoderm, and the two primary germ layers are only sharply distinguished from each other by their position.

After the egg has divided into four macromeres, micromeres begin to separate off from these at the formative pole. The separation of new micromeres from the surface of the macromeres continues, gradually extending from the formative towards the nutritive pole, and giving rise to the ectoderm, or single outer layer of cells, which gradually covers up all the surface of the macromeres except the small area of the blastopore. The formation of micromeres still goes on until the remnant of the macromeres is entirely surrounded by a single layer of cells, but as there is no more room on the surface of the embryo the micromeres which are last formed can arrange themselves in a single layer only by pushing inwards, and thus a primitive digestive cavity is formed and opens externally through the blastopore. If the Fresh-water Pulmonates could be considered by themselves, the formation of the two layered gastrula would thus admit of an extremely simple mechanical explanation.

If an embryo at this stage be carefully watched as it rotates, a second profile view, Figure 26, will be found with nearly the same

outline as Figure 24, but quite different in other respects. Careful examination shows that this is a view directly opposite the blastopore. The central mass, *en*, is not divided into a transparent and an opaque portion, and no central cavity can be seen. The segmentation cavity, *p*, is much more conspicuous than in the opposite view, and the ectoderm of its roof is sharply defined except at the sides, where the mesoderm, *m*, unites it to the central mass.

On the surface of the body, opposite the segmentation cavity, and, therefore, at right angles to the blastopore, there is a depression or invagination of the ectoderm, *mo*, which is, in profile view, a shallow pit, the definitive mouth. It has no connection with the central mass, but has a definite internal outline, and is simply a fold of the ectoderm. It soon changes its position, and in Figure 27 (*Planorbis parvus*, side view), the definitive mouth, *b*, and the blastopore, *e*, are at opposite poles of the body, and the foot, *o*, has begun to project between them. The change of relative position seems to be due to the crowding of the definitive mouth towards the anterior end of the body by the growth of the foot.

A great change has also taken place in the granular mass. The peripheral portion has become divided up into a number of large spherical, highly refractive, slightly transparent cells, *h*, which are arranged so as to form a layer or sheath around the smaller, more transparent endoderm cells, which deeper focusing brings into view.

Figure 29 is a similar view of a somewhat older embryo of *Physa*. In this figure, *i, i, i*, are the large, highly refractive cells which have been formed by the division of the opaque ring of Figures 24 and 25, and *k*, the layer of endoderm cells. At the stage shown in Figure 24, the opaque mass was close to the blastopore, but in 27 and 29 the invagination neck has lengthened and carried the central mass away from the blastopore and towards the mouth. Meanwhile, the cavity of the invagination neck becomes entirely obliterated, thus converting the "neck"; Figures 27 and 29, *g*, into the "rectal plug" of Ray Lankester. At the outer end of this plug, the blastopore is still represented by a slight depression, *e*, Figure 27, on the surface of the body. The mouth, 27, 28 and 29, *p*, is almost directly opposite this point, and is now a well-marked pit with a wide external opening. The layer of ectoderm which forms its wall, Figure 29, *p*, is pushed in

towards the central mass, but is not yet united to it, so, at this time, the digestive tract has no external opening.

In a front view of the stage shown in Figure 27, that is, a view of the right hand surface of this figure, the outline, Figure 28, is much like that of Figure 26, except that the mouth, *b*, is almost in the centre, and the foot, *c*, now occupies the position occupied by the mouth at the stage 26. On each side of the mouth there is an ear-like fold of the surface of the body, fringed with cilia, the folds of the velum. In Figure 29, they are united above the mouth on the median line, at *v*, and their free ends run out onto the sides of the body and bend upwards towards the head vesicle, but do not reach the dorsal surface of the body. At this stage, the blastopore is indicated by a slight depression, *e*, and the surrounding ectoderm is converted into a thick circular pad, *h*, with a flat outer surface, the shell area.

In an optical section of the embryo shown in Figure 29, the integument is made up of two layers, the ectoderm and mesoderm.

Figure 30 is an optical section of a somewhat older *Physa* embryo, in the same position as Figure 29. At this stage the foot, *c*, is quite conspicuous, and contains a cavity, *c''*, which is part of the body cavity.

By the growth of the foot the mouth has been pushed still farther from its primitive position, and a line drawn from the blastopore to the point which the mouth occupied in Figure 29, would have the mouth on its anterior and the foot on its posterior side.

This change has been accompanied by a decrease in the relative size of the head vesicle, *a*, which no longer occupies the whole dorsal surface as it did in Figure 29. The body of the embryo has lengthened, and the mouth-invagination has become a deep, thick-walled pouch, *c*, which runs inwards and downwards, and is divided by a constriction into the proper mouth cavity, *b*, and the pouch for the lingual ribbon, *li*.

The large refractive spherules, *m*, which are both relatively and actually much larger than at an earlier stage, have been pushed aside by the mouth-invagination, and only a few of them are visible in a median section. The internal surface of the mouth-invagination is in contact with the endoderm, although there is as yet no communication between its cavity and that of the stomach.

The blastopore is still represented by a notch, *e*, in the centre of the shell area, *h*, *h*, which is now a well-defined circular patch made up of several layers of cells.

From the centre of the inner face of this area, the rectal plug, *g*, runs inwards and becomes continuous with the endoderm of the digestive tract. The digestive cavity is quite conspicuous, but it cannot be traced into the rectal plug, which appears to be solid.

The mesoderm has now become well developed, and forms a single layer of rounded granular cells, *a'*, *o'*, lining the ectoderm of the foot, the head vesicle and the shell area, and thus forming a two-layered integument. On the sides and the lower border of the mouth the mesoderm bends inwards with the ectoderm, and may be seen as a distinct layer of granular cells covering the buccal mass, and spreading over the large spherules until it meets the mesoderm of the shell area and rectal plug.

No corresponding layer is present at this stage upon the upper surface of the buccal mass, or on the dorsal surface of the mass of large spherules.

Running from the mesoderm of the integument to that of the buccal body and central mass are a number of irregular branched processes, *o*, which are contractile. Although only a few of these processes lie in any one plane, they are much more numerous than the figure seems to indicate, and divide the body cavity into a system of sinuses; the processes are most numerous in the foot and the head vesicle, and these structures are now quite contractile, swelling up at times and projecting from the surface of the body, and then becoming almost flat. Opposite the head vesicle in this and in younger embryos, there is a space, *d*, Figures 29 and 30, where the foot joins the body, characterized by the thinness and great contractility of the integument. Among the processes of the mesoderm, a few detached corpuscles, Figure 30, *bl*, are kept in motion by the contraction of the body wall.

A surface view of an older embryo is given in Figure 31, and a longitudinal optical section of another of about the same age in Figure 32. To facilitate comparison with the adult they are figured with the shell area and shell above, the head vesicle to the right and the foot below.

While these two figures represent about the same stage of development they differ considerably in outline. This difference is due to the fact that the embryo now changes its shape continually by contractions of the integument.

Among the changes which will be noticed at this stage are first, the connection of the mouth invagination with the stomach, so that



the latter now communicates with the exterior; second, the migration of the distal end of the rectal plug from its primitive position to a point on the posterior surface of the body just outside the shell area, where it unites with the integument to form the anus; third, the appearance in the rectal plug of the intestinal cavity; fourth, the secretion of a symmetrical circular shell upon the surface of the shell area, and the thickening of the margins of this area to form the mantle-ridge; fifth, the appearance of the rudimentary tentacles inside the two bends of the velum; and sixth, the appearance of the forked structure which is described by Rabl as the nervous system, and by Stiebel (*Ueber die Entwicklung der Teichhornschnecke*, 1816) as the œsophagus and rectum. The position, shape and relations of this structure are correctly described by Rabl, but I was not able to satisfy myself as to whether it is or is not the nervous system. Each of the branches of the fork is tubular and its cavity is ciliated.

With respect to the later history of the embryo, and the formation of other organs than the digestive tract, I have nothing to add to the observations of Rabl and Lankester.

Going rapidly a second time over the ground covered by the previous description the following points present themselves.

1st. The polar globules make their appearance at what is to become the formative pole of the egg, and they mark the plane of the first cleavage.

2d. After the egg has divided into four equal spherules the protoplasm of each spherule undergoes a process of segregation; that which occupies the formative pole, and which is destined to give rise to the ectoderm, becoming quite transparent, while that which occupies the nutritive pole is opaque and granular.

3d. The formative ends of the four primary spherules separate as four micromeres, while the nutritive ends become converted into four much larger opaque macromeres.

4th. During the entire process of segmentation there is a very conspicuous alternation of periods of segmenting activity with periods of repose, during which the egg tends to become spherical, and the segmentation spherules become more or less fused together.

5th. By the division of the micromeres, and by the separation of others from the formative ends of the macromeres, a layer of cells, the ectoderm, is formed, and at last entirely covers the four

macromeres except at a point, the blastopore, which is at the nutritive pole of the egg, and directly opposite the polar globules.

6th. The four macromeres now become fused together and form an oval granular mass of protoplasm, part of which separates off in such a way as to form a layer of cells, the endoderm, arranged around a primitive digestive cavity which opens externally at the nutritive pole of the egg through the blastopore, around the margins of which the ectoderm becomes continuous with the ectoderm.

7th. After the separation of the endoderm the remainder of the granular mass divides up into a number of large cells, which are situated in the body cavity between the ectoderm and the digestive tract.

8th. These large cells are not to be regarded as a food-yolk for they grow with the growth of the embryo, instead of diminishing in size, and they soon become united to the surface of the endoderm by a layer of mesoderm, and they appear to give rise to the liver of the adult.

9th. There is no continuous embryonic middle layer, or mesoderm, but the layer of mesoderm which at the later stages of development lines the inner surface of the integument, and covers the digestive tract, appears to originate by the migration of mesoderm corpuscles from the margins of the blastopore, in almost precisely the manner which Selenka figures and describes in *Holothurians*, and *Echini*.

10th. The neck which unites the digestive tract to the blastopore, now lengthens; its cavity becomes obliterated, and the ectoderm cells about its outer end become converted into the shell-area, upon which the circular, symmetrical embryonic shell soon makes its appearance, and covers the region which the blastopore had occupied.

11th. The mouth originates as an independent invagination of ectoderm, and makes its appearance on the ventral surface of the body, about  $90^\circ$  from the blastopore, but is soon pushed, by the growth of the foot, to a point almost directly opposite the latter opening.

12th. It pushes in towards the digestive tract, but does not unite with it until after the blastopore has closed, and become covered by the shell.

13th. The cavity of the stomach appears to be a persistent portion of the primitive digestive cavity.

14th. After the "rectal plug" has been formed by the lengthening and closure of the invagination neck, its peripheral end moves from its primitive position in the centre of the shell-area, to a point on the ventral surface, between the shell and the foot; here it unites with the ectoderm to form the definitive anus, and an axial cavity, which now makes its appearance, converts the rectal plug into the intestine.

15th. The structure and history of the shell-area and velum are substantially as they have been described by Ray Lankester, and I have nothing to add to his account of the later history of the embryo.

#### DISCUSSION OF THE LITERATURE OF PULMONATE DEVELOPMENT.

Since the older papers on the development of Pulmonates give very incomplete accounts of the early stages, and contribute no information upon the history of the digestive tract, no notice of them is called for here; but the following papers, which contain observations upon the subject, demand discussion:

- 1st. Beitrag zur Lehre von den embryonalen Blätter bei den Mollusken. By M. Ganin.  
Warschauer Universitätsberichte. 1873, pp. 115-171.  
Abstract by Hoyer, in Hoffmann u. Schwalbe's Jahresberichte. I. 1872, p. 35.
- 2d. Observations on the Development of the Pond-Snail (*Lymnaeus stagnalis*), and on the Early Stages of other Mollusca. By E. Ray Lankester, M. A., Fellow and Lecturer of Exeter College, Oxford.  
Quart. Mic. Journal., 1874, XIV, N. S., pp. 385-391.  
Plates XVI-XVII.
- 3d. Der Ontogenie der Süsswasser-Pulmonaten. Von Carl Rabl.  
Jenaische Zeitschr., IX, 2, May, 1875, pp. 195-240, Taf. VII-IX.
- 4th. Ueber die Entwicklungsgeschichte von *Helix*. Zugleich ein Beitrag zur vergleichenden Anatomie und Phylogenie der Pulmonaten. Von Dr. Hermann von Jhering.  
Jenaische Zeitschr., 1875, IX, iii, pp. 299-317, Taf. XVII-XVIII.
- 5th. Ueber die Ontogenie von *Cyclas* und die Homologie die Keimblätter bei den Mollusken. Von Dr. H. von Jhering. Zeit. f. wiss. Zool., März, 1876, XVII, pp. 414-433.

6th. Sur le Développement des Gasteropodes pulmonés. H. Fol. Comptes Rendus, 1875, 81, pp. 523-6.

The second and third of these papers, that by Lankester and that by Rabl, contain observations and conclusions regarding the origin of the digestive tract which do not, to say the least, directly contradict my own. Neither of these authors succeeded in obtaining a satisfactory series of the early stages of development, and neither of the papers is entirely free from errors, but in the main, they furnish evidence which may be regarded as corroborative of my results.

Although my notice of these papers must necessarily dwell upon the points upon which I believe them to be incorrect, I should be very sorry to convey in this way the impression that they are generally inaccurate, for each of them is a valuable contribution to our knowledge of Pulmonate development, based upon careful, but by no means exhaustive or absolutely accurate, observations.

The first, fourth, fifth and sixth papers on the list are, as far as the history of the digestive tract is concerned, totally irreconcilable with my own observations, or with those of Lankester and Rabl; nor do they agree among themselves upon most of the points at issue.

For the sake of clearness, I shall divide my remarks on the literature into sections, and speak first of the views of the process of segmentation, and the formation of the gastrula, then of the history of the change from the primitive digestive tract into the permanent one, and then of certain more general questions.

Lankester's account of the process of segmentation, and of the formation of the gastrula, is confessedly incomplete, and although the stages which he Figures (Plate XVI, Figures 1-15), are, with two exceptions, accurately represented, they are too isolated to contribute any information as to the origin of the germ layers of the segmenting egg.

In Figures 4 and 5 he represents the polar globules at the nutritive pole of the egg, but, as I have already pointed out, he states his doubts as to the accuracy of this, certainly erroneous, observation.

Lankester is the only observer, so far as I am aware, who has noticed the flattening of the egg at the close of segmentation, as I have shown it in Figure 20.

His Figures 10 and 11, Plate XVI, represent a nearly similar stage of development, and on page 377 he says: "The gastrula of *Lymnaeus* has the same curious cushion-like form which I have observed in the Nudibranchs."

His next Figure, 12, seem to be a side view of about the stage shown in Figure 24 of the present paper, but he overlooks the small endoderm cells which, at this period, line the primitive digestive cavity and separate it from the remainder of the granular mass.

Rabl's figures have by no means the same accuracy. His account of the early stages of segmentation, p. 198, and his figures of these stages, Taf. 7, Figs. 1-7, are precisely like mine, but his account of the manner in which the gastrula stage is reached, pp. 198-199, as well as his figures of the morula and the gastrula, Figures 8-12, are certainly diagrammatic. My own observations justify the statement that these figures do not give the actual appearance of the embryo at these stages, and my own statement that the primitive gastrula is flattened instead of long, worm-like, and divided into three segments, as in Rabl's description, and his Figure 12, is confirmed by Lankester's observation already quoted.

As regards the main point of the question Rabl agrees with Lankester and myself in asserting that segmentation results in the formation of a true gastrula, with primitive digestive cavity and external opening.

Fol's testimony is to the same effect, but Ganin and von Jhering give an entirely different account.

I have not seen Ganin's original paper and my acquaintance with it is through the very ample and satisfactory summary by Hoyer in Hoffmann and Schwalbe's *Jahresberichte*. While this review or notice gives what appears to be an adequate statement of Ganin's views it is not accompanied by figures, and although I believe Ganin to be in error I cannot, in the absence of the original paper, attempt to give any explanation of the manner in which the error has originated.

According to his view the process of segmentation results in the formation of a single layer of somewhat similar cells, surrounding a central cavity. Over one hemisphere of this nearly spherical embryo the cells divide more rapidly than they do at the other, and one pole of the embryo, the dorsal pole, thus comes to be made up of large, dark, granular cells, while the opposite pole is occupied

by a layer of small, flattened, more transparent cells. At a somewhat later stage, after various organs have made their appearance, the layer of smaller cells grows over and entirely covers the large cells, which are thus carried into the cavity of the embryo, and become a food yolk; while the outer layer of cells is regarded as a blastoderm. At first the blastoderm is only one cell thick, but soon a portion of it, on the ventral surface, and therefore opposite the point on the dorsal surface where it closed in over the food yolk, becomes several cells thick, and the cells of this thickened area soon divide into three layers, the ectoderm, the mesoderm and the endoderm.

The inner layer, or endoderm, pushes in towards the food yolk, and gives rise to the digestive cavity, and its outer end unites with the ectoderm, on the ventral surface, where an opening, the mouth is formed. The intestine grows out from its posterior end towards the body wall, with which it finally unites to form the anus. The middle layer splits, and thus gives rise to the body cavity, and the outer one of the two layers formed by the split unites with the ectoderm to form a somatopleur, while the inner one unites with the wall of the digestive tract, to form a splanchnopleur.

It is clear that this account cannot be reconciled with those of Lankester, Rabl and Fol, or with my own observations, but it is useless to speculate, in the absence of figures, concerning the facts which have led Ganin to this interpretation. That it is erroneous is shown, not only by the later papers on the development of Pulmonates, but by all the recent papers on the development of Molluscs. Von Jhering gives still another view of the process of segmentation and of the origin of the digestive tract. As the full statement of his conception of the nature of the process is contained in his paper on *Cyclas* I shall discuss it in a paper, which is now in press, on the development of Lamellibranchs, and will only give it a brief notice here.

He says (*Ontogenie von Cyclas*, p. 425), that in all the Platycochlida (The Nudibranchs, Pulmonates, Tectibranchs, Pteropods and Cephalopods) "Die Furchung eine inequale ist, und die kleinen formativen zellen die grossen nutritiven umwachsen, und von den so gebildeten beiden primären Keimblättern wesentlich nur das äussere sich an dem Aufbau des Körpers theilnimmt, indem das primäre Entoderm ganz oder grossentheils der Resorption anheimfällt." He says that Rabl's paper is the only one which

tends to show that any of the *Platycochlida* pass through a gastrula stage, and doubts whether Rabl has seen such a stage. He concludes that, if Rabl's observations are incorrect, as he supposes, "Dann reiht sich *Limnaeus* ganz in die onto-genetische Kategorie ein, welche allen *Platycochliden* gemeinsam zu sein scheint, und es ist weder bei den *Platycochliden* noch auch bei den *Lamelli-branchien* der Vorkommen der Invaginationsgastrula bis jetzt in irgend einer Falle nachgewiesen."

The scientific worthlessness of this generalization hardly needs comment, for, among the representatives of the so-called phylum *Platycochlida*, Fol has shown that the *Pteropods* go through a true gastrula stage, and that there is nothing like von Jhering's *Leposphere* stage. Lankester has shown that the same is true of certain *Nudibranchs*, and both of these writers have, like Rabl, asserted the fact of the occurrence of the gastrula stage in the *Pulmonates*.

I think that this short review of opinions and observations regarding the earlier changes of the *Pulmonate* embryo is sufficient to show that the evidence which we possess is not on the whole opposed to my own observations. The evidence of Lankester, Rabl and Fol tends to support my view, so far as it is opposed to the statements of Ganin and von Jhering; the points in which my account of the formation of the gastrula differs most essentially from the account by Rabl, are substantiated by Lankester, and the points upon which the last writer and I disagree are those upon which he confesses that his observations are incomplete and unsatisfactory.

As regards the later stages in the development of the digestive tract, the accounts by Rabl and Lankester agree, in the main, with my own, although neither of them is complete, and neither of them has observed exactly the same points, so that each account resembles my own much more than they resemble each other.

Rabl correctly describes the mouth, p. 203, as an invagination of the ectoderm, which has at first no connection with the digestive cavity. He figures it correctly in a front view, Figure 17, but in a side view, Figures 18 and 19, he confuses it with the almost obliterated primitive opening in the centre of the shell area. In Figures 20-23, it is again shown correctly, and I think a comparison of my figures with his will show clearly that he is in error as to the orientation of Figures 18 and 19. As regards

the fate of the gastrula mouth, he says that, although he was not able to trace the transformation of the primitive gastral opening into the anus, yet such a transformation appears more probable than its change into the definitive mouth. The late formation of the anus appears to him to oppose this view of its origin, however, for it would be expected to be present before the definitive mouth. He correctly describes the way in which the true endoderm of the digestive tract splits off from the inner surface of the thick invaginated layer, and thus leaves part of this layer inside the body cavity; but he regards this latter portion as a food-yolk, and says that it gradually disappears. He also says that the primitive digestive cavity is entirely filled up and obliterated by the formation of the true endoderm, and that a new cavity is then formed by the separation of these cells from each other.

So far as the figures of later stages which are given in Lankester's second plate, Plate XVII, Figures 1-23, relate to the subject of the present paper I have few corrections to make.

In Figure 1, he correctly shows the mouth *m*, as an independent invagination, opposite the primitive invagination, *g*, which is now closed, and situated at the outer end of the rectal plug, *pi*. In this figure he indicates another area, *sp*, as the rudimentary shell patch.

According to my observations the area, *g*, of Lankester's Figure 1, is the shell area, and the position of the rectal plug and anus outside this area, as correctly shown in his Figures 4 and 11, is brought about by a change which he has overlooked: the migration of the distal end of the rectal plug from its primitive position. His description of the formation of the digestive tract leaves many important points unsettled, but so far as it is based upon observation it agrees with my own account.

In order to show this essential agreement, as well as to call attention to the many points which he leaves undecided, I shall quote from his account at some length.

He says, p. 383: " \* \* \* It must be admitted that to follow out fully the development of the alimentary canal is exceedingly difficult, even as far as its general contour is concerned; still more so when a histological and histogenetic point of view is attempted. In fact, here, as in all the embryologies which have been attempted, the dark point is in connection with the middle portion of the alimentary canal. If we knew with certainty when and how its cellular elements are developed in all types which have been



studied, we should have little difficulty in reducing the facts of development of the whole animal kingdom to satisfactory order. We have seen that there results from the gastrula-invagination an outer cellular body-wall, from the elements of which the epidermic and muscular structures of foot, velum, mantle, and shell-gland develop, and an inner invaginated sac composed of larger cells, supported on a short pedicle, (the cells of which are not large and granular, but scarcely distinguished,) the pedicle of invagination. \* \* \* The pharynx now commences to develop with the inpushing of the mouth from the body-wall, and gradually extends downwards into the mass of endoderm cells, so as to be partly concealed by them. At the same time, the cells in the pedicle of invagination differentiate. The pedicle assumes a tubular character, and its parietal end becomes bent round, so that the tube terminates as a shortly reflected coecum."

At this point I omit the author's account of the mesoderm corpuscles which are shown in my figures, as gradually spreading over the digestive tract, and also forming a mesodermic lining to the integument.

Lankester is inclined to believe that those cells of this layer which cover the alimentary canal are derived from its surface, while those which line the integument are derived from the ectoderm. He gives no observations to support this view, which is given merely as a conjecture.

He continues, pp. 384 and 385: "We have to see what eventually becomes of the middle group of cells. \* \* \* I have failed to penetrate to the centre of this mass of cells in earlier phases, and can, therefore, not explain how the structure, to be described, comes about. What can be observed is this, that as soon as the pharynx and its appendix, the odontophore's sac, becomes well marked, and the tubular structure with epithelial lining in the pedicle of invagination is clearly visible, then a little compression and manipulation renders clear the continuation of a tube-like structure with walls formed of small cells from the pharynx to the intestine, traversing the mass of large pellucid cells. This tubular structure is undoubtedly to be regarded as the so-called stomach of the adult Lymnaeus.

"The metamorphosed gastrula-endoderm-cells now lie on each side of it as a pair of grape-like bunches, and long after it has become well defined, these two agglomerations of pellucid spheres,

with their enclosing network and mesoblastic coat, remain. They are apparently eventually absorbed as nutritive matter by diverticula of the alimentary canal which give rise to the liver, they themselves not giving rise subsequently to any permanent tissue.

"Now it is a most important question whether the cell-elements which build up the so-called stomach, the middle piece of the alimentary canal, arise in any way from the large gastrula-endoderm-cells, or from the pharyngeal inpushing, or from the intestinal pedicle of invagination. If from this last, they would just as much, as if they arose from the central mass of gastrula-endoderm, be traceable to the invaginated cells of the gastrula-phase.

"On the whole, it seems probable that this is their origin, but the matter is still obscure."

This last sentence is also a little obscure, but if the word *this* is intended to refer to the words *gastrula endoderm* of the preceding sentence, it will be seen that Lankester's observations lead him to incline towards the view which Rabl's observations and my own have shown to be the true one.

It will be seen from the above extracts that the only point upon which my observations directly contradict those of Lankester, concern the relative positions of the primary invagination and the shell gland, and my series of embryos seemed to furnish such conclusive evidence that the shell area occupies the same place as the invagination, that I feel confident of the correctness of my own statement.

As regards the conversion of the pedicle of invagination into the intestine, and the independent formation of the mouth by an entirely distinct invagination of the ectoderm, his account agrees with my own.

Before closing, I wish to say a few words which are not connected with the history of the digestive tract. Lankester devotes considerable space to a description of the Pulmonate velum and the embryonic shell. Rabl's entirely independent observations, published a little later, contain figures and a description of the same structures, and fully corroborate all of Lankester's observations. Although I have not devoted much space to this subject, it will be seen that my figures also testify to the correctness of Lankester's account.

Von Jhering's paper on the Development of Helix, which was published nearly simultaneously with Rabl's paper, contains a

violent attack upon Lankester's account of these organs, and is full of statements which can only be due to wilful misrepresentation, or to an inability to correctly translate the English paper.

On pages 307-308, he describes and figures (Figures 7, 12, 13) the velum of *Helix*, and so far as I am able to perceive, the organ which he describes agrees with the velum of *Limnaeus*, as described by Lankester, in every essential characteristic, but the author holds that Lankester has failed to discover the actual velum, and that the structure which he describes is the ciliated ring-shaped ridge formed by the edge of the mantle.

On pages 310-311 he describes the shell-area of *Helix*, substantially as Lankester has figured and described it in *Limnaeus*, except that he says the shell of *Helix* is internal at first, and that there is no groove in the shell-area. In his later paper on the Development of *Cyclas*, he says p. 417, that the second of these points is an error, and in a foot-note to the same paper No. 4, p. 417, he owns that he was also wrong in the statement that the shell of *Helix* is at first internal.

After his description of the shell-area of *Helix*, he says: "Die Mantelanlage hat in neuester Zeit zu einem eigenthümlichen Missverständniss Anlass gegeben. Die betreffenden Angaben gleichfalls in der schon citirten Abhandlung von Ray Lankester's enthalten, sind, für Jeden, der nicht die erstauenswerthe Unkenntniss und zugleich die zügellose Phantasie des Autors jener wunderbaren Abhandlung besitzt, so unfasslich und der Wiederlegung unwerth, dass nur der Umstand mich veranlasst sie überhaupt zu berücksichtigen, dass Ray Lankester für die Bestätigung seiner Ansichten ausdrücklich an die Erfahrungen späterer Schriftsteller über Pulmonaten-Entwicklung appellirt."

"Ray Lankester hält nämlich die Byssusdrüse der Acephalen resp. ihr Sekret nicht nur für die erste Anlage des Schalenligamentes der Muscheln, sondern er lässt sie auch bei *Limnaeus* unter der Schale, beziehungsweise dem Mantel existieren, Grund genug für ihn, um nun die innere Schale von *Limax* mit ihr, seiner Shellgland für homolog zu erklären."

As von Jhering confesses in another place that he was wrong in the only points regarding which his description of the shell area differs from Lankester's, his attack upon Lankester seems to be a little severe, and the same motive which he says induced him to make it, Lankester's appeal to later writers, has led me to call attention to it again, by the quotation which I have given.

## EXPLANATION OF THE FIGURES.

All the figures were drawn as seen with a magnifying power of 250 diameters; Zeiss, Ocular 2; Objective D.

Figures 1, 2, 3, 4, 5, 6, 19, 20, 21, 22, 23, 24, 25, 26, 29, 30, 31 and 32, are stages in the development of *Physa heterostropha*, while figures 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 27 and 28 are from the eggs and embryos of *Planorbis parvus*.

FIGURE 1.—Freshly laid egg of *Physa heterostropha*.

FIGURE 2.—The same egg, a few minutes later.

- a. Vitelline membrane.
- b. Transparent space between this membrane and the yolk.
- c. Transparent area at the formative pole of the egg.
- p. Polar globules.

FIGURES 3, 4, 5, 6.—Successive stages in the primary segmentation of the same egg, figured at intervals of about ten minutes.

- p. Polar globules.

FIGURE 7.—Optical section of egg of *Planorbis parvus*, during the period of rest which follows the first period of active segmentation. The two primary segmentation-spherules are apparently fused together peripherally, but separated from each other centrally by a lens-shaped segmentation cavity.

FIGURE 8.—View of the formative pole of the egg of *Planorbis parvus*, at the end of the second period of active segmentation, showing the division into four spherules.

- A, A. Primary spherules.
- B, B. Secondary spherules.

FIGURE 9.—View of the nutritive pole of the same egg, during the second period of rest, about five minutes after the stage shown in Figure 8.

- A, A. Primary spherules.
- B, B. Secondary spherules.

FIGURE 10.—Optical section of the same egg, showing the segmentation cavity.

**FIGURE 11.**—Side view of an egg which is about to divide into macromeres and micromeres.

In this, and all the following figures of segmentation, the egg is shown in side view, with the germinative pole above.

**FIGURE 12.**—The same egg, about fifteen minutes later, at the end of the third period of activity.

*A, A, A, A.* The four macromeres at the nutritive pole.

*a, a, a, a.* The four micromeres at the formative pole.

*p.* Polar globules.

**FIGURE 13.** The same egg, about ten minutes later, during the early part of the third period of rest.

*A, A, A, A.* Macromeres.

*a, a, a, a.* Micromeres.

*p.* Polar globules.

*sg.* Segmentation cavity.

**FIGURE 14.**—Optical section of the same egg, about ten minutes later, at the end of the third period of rest.

*A.* Macromeres.

*a.* Micromeres.

*sg.* Segmentation cavity.

*n.* Nuclei.

*p.* Polar globules.

*g.* Accumulation of opaque granular matter at the nutritive ends of the four macromeres.

**FIGURE 15.**—Optical section of the same egg during the beginning of the fourth period of active segmentation.

*A, A.* Macromeres.

*g.* Granular matter at their nutritive ends.

*a, a.* First set of micromeres.

*p, p.* Second set of micromeres.

*p.* Polar globules.

*sg.* Segmentation cavity.

**FIGURE 16.**—Optical section of the same egg, at the end of the fourth period of active segmentation.

Letters as in Figure 15.

**FIGURE 17.**—Optical section of the same egg at the end of the fourth period of rest.

Letters as in Figure 15.

FIGURE 18.—Surface view of the same egg during the period of rest which follows the fifth period of active segmentation. The egg is now divided into sixteen spherules, divided into four sets of four each.

- A, A.* Macromeres.
- a, a.* First set of micromeres.
- b, b.* Second set of micromeres.
- c, c.* Third set of micromeres.
- p.* Polar globules.
- g.* Granular matter at nutritive end of macromeres.

FIGURE 19.—A surface view of a somewhat older egg, divided into thirty-two segments, and figured at the end of a period of division of the nutritive end.

- p.* Polar globules.
- ec.* Ectoderm.
- A.* Macromeres.

FIGURE 20. Primitive gastrula stage of *Physa heterostropha*, viewed from one side, and with the formative pole above, showing the four large opaque macromeres covered by an ectoderm of much smaller transparent cells. This figure is in the same position as the preceding ones, the top being the formative pole of the segmentating egg.

FIGURE 21.—The same stage of development viewed from the nutritive pole.

FIGURE 22.—A somewhat older embryo of *Physa heterostropha*, viewed from the side, or in the same position as Figure 20, showing the fusion of the four macromeres into a continuous mass, and the formation of a body cavity between this mass and the ectoderm. In copying this figure for photography, the ectoderm cells have been drawn too large.

FIGURE 23.—The same stage of development, viewed from below.

- ec.* Ectoderm.
- g.* Blastopore.
- h.* Body cavity.

FIGURE 24.—A somewhat older *Physa* embryo, in the same position as Figure 23.

FIGURE 25.—Optical section of the same embryo.

- ec.* Ectoderm.
- b.* Body cavity.
- m.* Mesoderm.
- f.* Granular mass.
- en.* Endoderm.

**FIGURE 26.**—The same embryo, viewed from the surface opposite that shown in Figure 25.

*mo.* Mouth-invagination.

Other letters as in Figure 25.

**FIGURE 27.**—A side view of a somewhat older embryo of *Planorbis parvus*.

*a.* Head-vesicle.

*b.* Position of mouth-invagination.

*c.* Foot.

*d, d.* Body cavity.

*e.* Position of Blastopore.

*g.* Rectal plug.

*h.* Layer of small endoderm cells, surrounded by a layer of much larger opaque, highly refractive spherules.

**FIGURE 28.**—The same embryo viewed from in front, that is, from the surface which is at the right hand in Figure 27.

*a.* Head-vesicle.

*b.* Mouth invagination.

*c.* Foot.

*d.* Body cavity.

*h.* Inner cells of body.

*v.* Velum.

**FIGURE 29.**—A *Physa* embryo, at a little older stage than that shown in Figure 27, but in the same position.

*a.* Head-vesicle.

*a'.* Its cavity.

*b.* Mouth-invagination.

*b'.* Mouth.

*c.* Foot.

*c'.* Cavity of the foot.

*d.* Contractile area at the posterior boundary of foot.

*e.* Position of the orifice of invagination.

*g.* Rectal plug.

*h.* Shell patch.

*i.* Large, highly refractive spherules.

*k.* Endoderm.

*v.* Velum.

**FIGURE 30.**—Median vertical optical section of a still older *Physa* embryo, in the position of Figure 29.

*a.* Ectoderm of head-vesicle.

*a'.* Mesoderm of head vesicle.

FIGURE 30—*Continued.*

- a''*. Cavity of head-vesicle.
- a*. Mouth-invagination.
- b', b'*. Edges of the mouth.
- li*. Pouch of lingual ribbon.
- c*. Ectoderm of foot.
- c'*. Mesoderm of foot.
- c''*. Cavity of foot.
- d*. Ectoderm of contractile area.
- d'*. Cavity of contractile area.
- e*. Position of orifice of invagination.
- g*. Rectal plug.
- h, h*. Margins of shell-gland.
- k*. Endoderm.
- l*. Digestive cavity.
- m*. Large spherules.
- n*. Inner layer of mesoderm.
- o*. Branching processes, connecting this layer with body-wall.
- v*. Velum.
- bl*. Blood corpuscles.

FIGURE 31.—Still older embryo of *Physa*; surface view.

- a, a', b, b', c, c', d*, as in Figure 30.
- e*. Shell.
- f*. Margins of mouth.
- g*. Anus.
- h*. Large spherules.
- i*. "Nervous system."
- v*. Velum.
- t*. Tentacle.

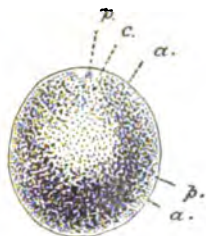
FIGURE 32.—Vertical median optical section of an embryo at the same stage as that shown in Figure 31.

- a, b, c, c', c'', d, d', e, f, h*, as in Figure 31.
- g*. Rectum.
- i*. Endoderm of stomach.
- k*. Endoderm of intestine.
- l'*. Endoderm of oesophagus.
- v*. Velum.
- li*. Lingual mass.
- l'*. Its cavity.
- l''*. Its mesoderm.
- bl*. Blood corpuscles.

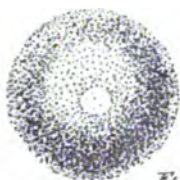


**Development of Pulmonates.**

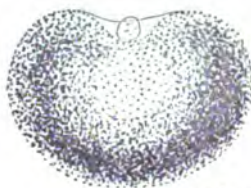
**Plate 1.**



*Fig 2.*



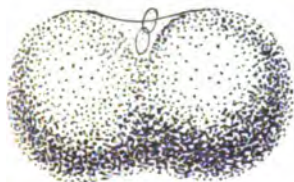
*Fig 1.*



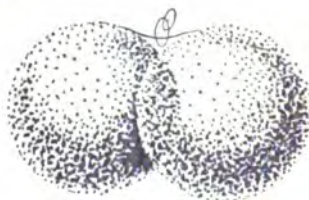
*Fig 3.*



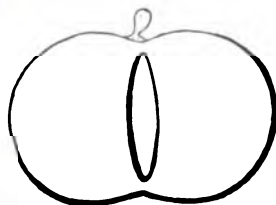
*Fig 5.*



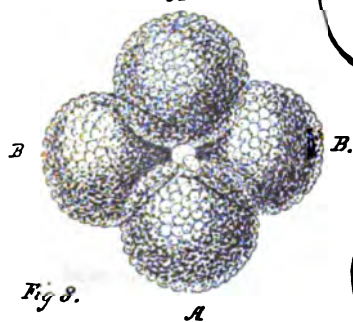
*Fig 4.*



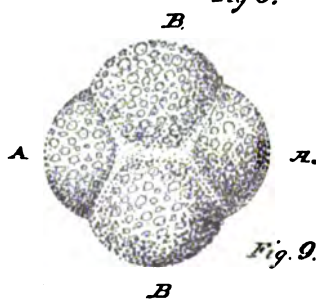
*Fig 6.*



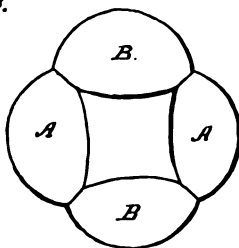
*Fig 7.*



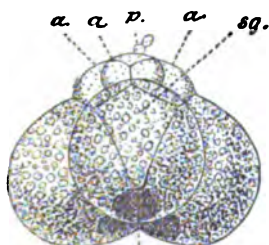
*Fig 8.*



*Fig 9.*

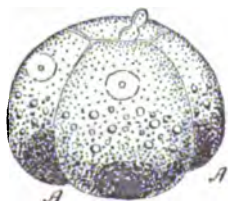


*Fig 10.*



*Fig 13.*

*W.K. Brooks. Del.*



*Fig 11.*



*Fig 12.*

1

# Development of Pulmonates.

## Plate 2.

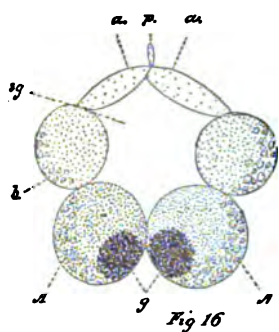


Fig. 16.

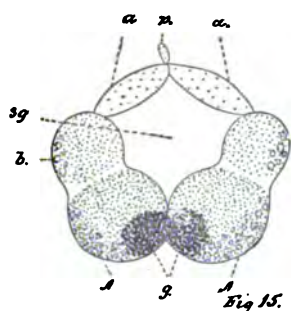


Fig. 15.

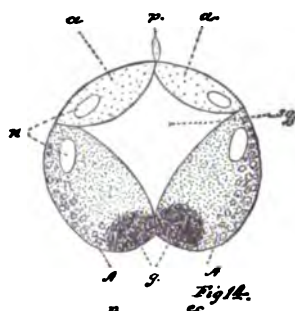


Fig. 14.

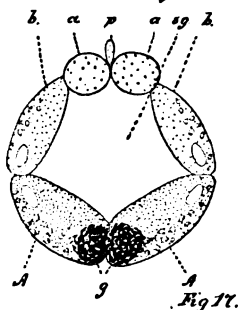


Fig. 17.

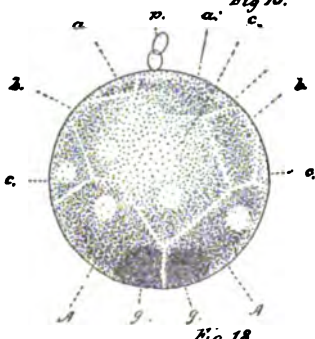


Fig. 18.

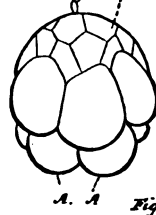


Fig. 19.

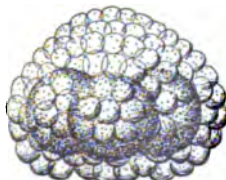


Fig. 20.

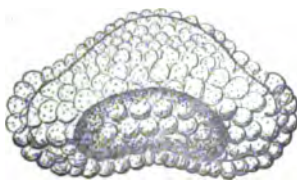


Fig. 22.



Fig. 21.

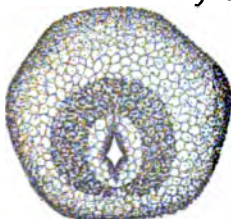


Fig. 24.

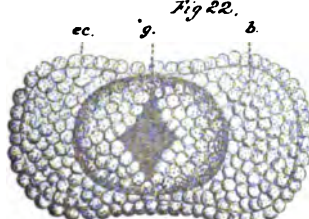


Fig. 23.

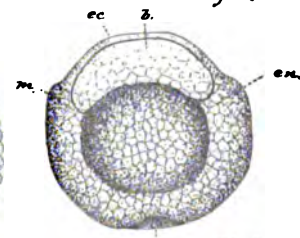


Fig. 26.

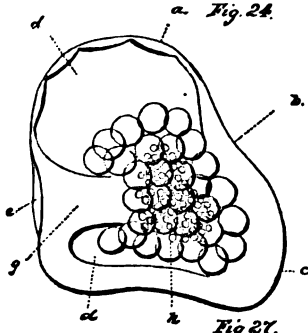


Fig. 27.

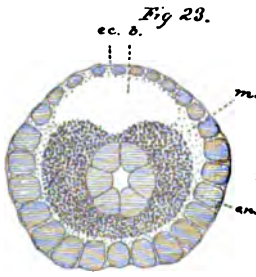


Fig. 25.

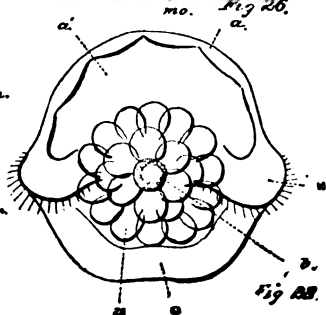
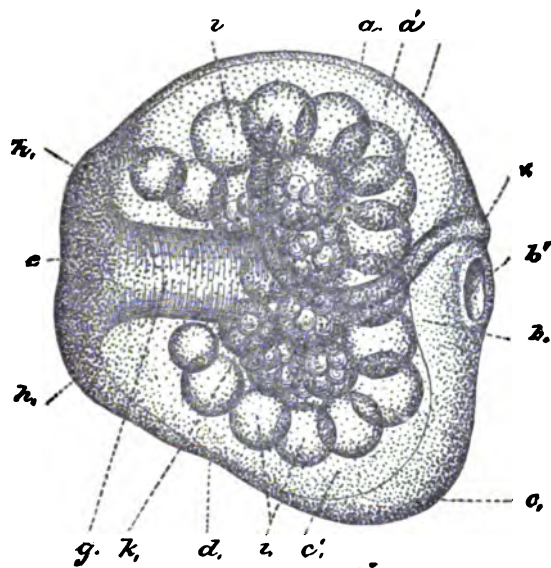


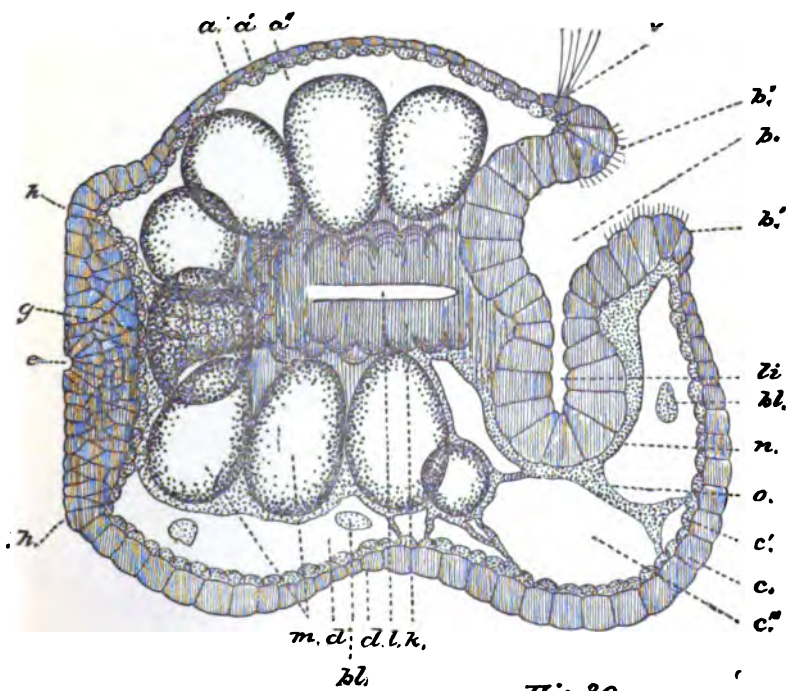
Fig. 28.

W. K. Brooks, Del.





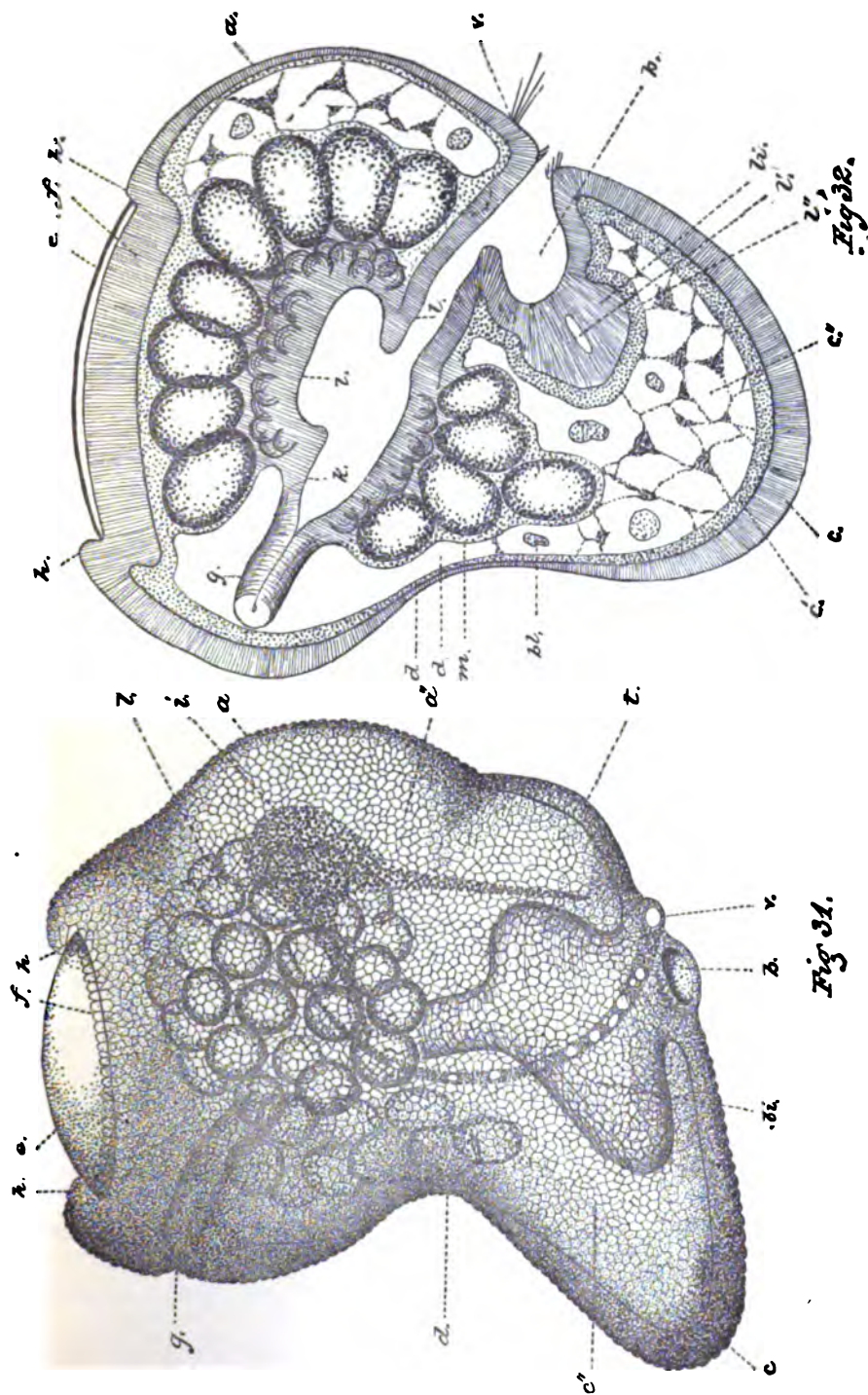
*Fig 29.*



*Fig 30.*











**THE DEVELOPMENT OF AMBLYSTOMA PUNCTATUM, Baird. PART I, EXTERNAL. BY SAMUEL F. CLARKE, PH.D., BALT., Assistant in the Biological Laboratory and sometime Fellow of the Johns Hopkins University.**

IN early March of 1878, I obtained in early stages of development a number of eggs which I believed to be those of some Urodele. They were found in considerable numbers in the pools and small streams in and near the woods about Baltimore, during the months of March and April. They occurred in gelatinous masses, Plate 4, Figure 30, which varied greatly in size, were usually more or less oval in shape, and attached to the stem of some aquatic plant or to an overhanging blade of grass.

This year I was so fortunate as to secure living specimens of both sexes of *Amblystoma punctatum* before the females had deposited their eggs. They all did well in confinement; the males furnished an abundance of spermatozoa at the critical moments, the eggs passed through their various phases of development, and a record of the external change is preserved by a series of camera-lucida drawings. The animals derived from the eggs brought to me in the spring of 1878, were studied with considerable care and received considerable attention in respect to their food and surrounding conditions. I was unable, however, to keep them after they reached the abbranchiate stage, and in consequence could not determine what form I had been at work upon. I was much pleased then to find upon carefully comparing the eggs laid by the adults in my aquaria this spring, their course of development and their more advanced forms, that they agree in every particular with the eggs and young forms which had occupied my attention in the previous years. By getting the eggs in this way I was able also to obtain the changes during segmentation, which I had not gotten from my previous observations.

The eggs, as I have said, occur in gelatinous masses, and these vary in size from a small bunch of three or four eggs to a large mass containing two hundred eggs and weighing sixteen ounces. When the eggs are deposited in the water they are seen to be covered with a very viscid, tenacious, translucent substance, of

which there seems to be but a thin coating, serving to keep together the eggs which have been deposited in that particular spot. This viscid substance however, rapidly absorbs water and in a few hours forms the bulky gelatinous matrix in which the eggs are contained. During the early stages of development the matrix is of sufficient consistency to hold together when the mass is suspended by a small portion held in the hand. As development progresses however, the mass at first gradually, afterwards more rapidly, loses consistency, so that by the time the embryos have acquired their balancers, the eggs will almost drop out of the matrix when the mass is held out of water in the hand. Each egg is surrounded by two membranous shells and the large space between the two membranes as well as the inner space, is filled with a clear, transparent fluid. The embryo thus situated within two elastic, spherical sacs containing fluid and the whole placed within a mass of yielding gelatinous substance, is well protected against injuries from collision with hard objects and also from becoming the food of voracious fish; for the latter appear to find no satisfaction in drawing into their mouths, portions of this gelatinous material which slips out as often and as rapidly as it is drawn in.

I was interested to find, after carefully watching the process a number of times, that the number of eggs deposited at a time depends upon accident. If the creature is disturbed, as by another individual striking against or touching it, or by the moving or jarring of the dish, she immediately suspends operations, and seeks some more quiet spot for the continuance of her labors. I have seen a single egg deposited and again a bunch containing one hundred and fifty. While the eggs are being extruded the animal usually lies with its anterior limbs extended laterally, while the hind limbs are curved around the opening of the cloaca and appear to assist in holding together the eggs as they are laid.

The males showed no inclination to clasp the females, but quietly deposited quite large masses of an apparently rather thick liquid, opaque white, on the bottom of the dish in which they were kept. Upon examination this liquid was found to consist of spermatozoa moving actively in a liquid. The eggs were found to have adhering to their outer shells, shortly after, a considerable number of these male elements, but I could not succeed after trying a great many times in finding any spermatozoa within even the outer shell.

Most of the eggs were laid during the night, and by nine o'clock the next morning the first segmentation furrow had usually made its appearance. The spermatozoas, Plate 4, Figure 31, are unusually large, averaging .75 millimetre or .03 of an inch in length. They are very slender and acutely pointed at both ends. When first thrown out they often have a remnant of the mother-cell still attached to some portion of them, but on account of their active movements it is soon thrown off. As active movements begin to cease in them, one end is often bent around till it touches and adheres to the body, thus forming a loop of variable shape and dimensions, which has much the appearance, until carefully studied, of an enlarged portion or "head" of the spermatozoid, Plate 4, Figure 31c, 31d. For ready reference, I give the measurements of the spermatozoa of a number of different amphibia, both Anoura and Urodela.

Rana temporaria, . . . . .	.008 to .011 of a mm.
Pelobates fuscus, . . . . .	.017 " "
Triton, . . . . .	.088 " "
Menopoma allegheniense, . . . . .	.25 " "
Amblystoma punctatum, . . . . .	.75 " "

The first three are taken from Wagner and Leuckart's article,\* the others were made by myself.

A few minutes after an egg is deposited there exists between the inner shell or membrane and the yolk, a quantity of gelatinous matter which seems to form, as development goes on, a third, inmost shell, very delicate and hyaline. The yolk lies so close to this inmost shell that it cannot at first be distinguished. As the process of segmentation begins, the yolk-mass is separated by a small space from this inmost shell, when the latter becomes distinctly visible. It remains until the medullary folds are nearly closed in, when it disappears; it being often, if not always, torn apart by the rapidly elongating embryo. At this early period the diameter of the outer shell is about twice that of the inner, and this relative size is maintained with considerable regularity throughout the period of intra-oval life. Both shells now rapidly increase as water is absorbed. By the end of segmentation the shells have reached nearly or quite their largest size, and remain as they then are until the embryo bursts them and makes its way out.

If a freshly laid egg be stripped entirely of its shells and all adhering gelatinous matter, it will be found to be divided into two zones which are almost exact hemispheres, marked out by colors. One hemisphere is black, and the other quite light, almost white. The light portion is not evenly colored; the lightest part of it forms a zone lying next to the dark hemisphere and the darkest portion of the light hemisphere is at the pole, the spot where the vitelline plug is to be formed. This coloration changes as development goes on. Although the lighter hemisphere is not a clear white, it is a sufficiently light color to make the two hemispheres quite sharply defined. It can readily be seen by pricking open an egg and allowing the contents to flow out, that this coloring matter lies on the surface, the inner contents of the egg being uniformly opaque—white. If one of these unfertilized eggs be placed in water, it instantly and always assumes a position with the dark hemisphere up and the light pole down; and as often as it is turned over in any other position it immediately rights itself when the retaining force is removed. As sections show no cavity in the eggs at this period it must be that the density of the unfertilized egg is not uniform and that the lighter colored is always the denser hemisphere. The cavity of Von Baer has not yet appeared, and that moreover would not, probably, be large enough to cause such a difference in density as is indicated by the quickness with which the unrestrained egg always takes this position. After the segmentation-cavity is formed, that portion of the spherical yolk containing this cavity is of course lightest and uppermost; but before segmentation and fertilization, when no cavity exists, this action must be produced by a difference in density of the particles composing the yolk. What can be the object of this arrangement by which the different colored poles are thus placed, it is difficult to conjecture. The darker colored areas would absorb more heat from the sun's rays, which under the usual natural conditions would be beneficial to rapid development. The arrangement is the same also as the protective coloring in many birds and fishes; the upper side dark and the under side light. This might be of some service to them, as fish of large size might eat small bunches of eggs and would attack them from below, as the egg-masses are usually at or near the surface. Goette says in respect to this coloring of Batrachian eggs—

"All the observers of the pigmented, developing Batrachian egg agree in this, that sometime after fertilization they turn themselves always with the dark pole upward, even if it was not the case at first. A sufficient reason for this cannot be found. According to my view, this turning of the yolk is apparent, whether general or in part, since only the pigmentary layer following the influence of the newly determined pole, displaces itself."\*

Segmentation commences by the appearance of a furrow on the dark hemisphere which stretches around the egg, the two ends meeting at the light pole, and thus dividing the egg into two hemispheres, each of which contains half of the dark and half of the light hemispheres. The two color areas during the early stages of segmentation are more distinctly outlined than at any other period. The dark area has become a rich dark brown. The second furrow forms a great circle at right-angles to the first, and starts also at the dark pole. After the formation of these four meridional sections, by the two furrows, a third furrow passes around the equator and separates the dark from the light hemisphere very sharply. The third segmentation furrow in Triton and in Bombinator differs from Amblystoma in being not equatorial, but nearer the upward pole. From this point segmentation progresses quite rapidly and at different rates in the two color areas; it being much more rapid in the lighter one. As the segments begin to get quite small, more and more color makes its appearance in the light area until as segmentation is about completed, only a small light area is left at the lighter-colored or downward pole. At the time when the first two furrows are complete there may be seen on the different segments near the light pole, a few small depressions in the substance of the yolk, the "trous vitellins" of Dr. Van Bambeke.† They soon disappear however, being visible for a few hours only. Upon examination, it is readily seen that segmentation progresses much more rapidly in the light hemisphere, and that it is carried on with very little regularity, in either. When the egg has finished segmentation the entire surface has become dark colored with the exception of a small irregular area surrounding the lighter pole and stretching away from it in one direction. The cells or segmentation-masses immediately

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\*Vide Der Unke, p. 58.

†Bulletins de l'Académie royale de Belgique, 2<sup>me</sup> série, Tom. XXX, Nr. 7, 1870.

about the pole are larger than the others. Very soon an irregular, slight depression of this polar region occurs, which lasts but a short time; this area becoming again even with the surface of the sphere. But this movement or action has resulted in the formation of a very narrow, even, clearly marked groove, which sharply defines, or makes a distinct boundary to, the polar portion of the white area, and extending on either side along the edge of that portion of the lighter area which stretches away from the pole, gradually fades out. See Plate 1, Figure 1. Now the curved groove becomes less and less widely open in front, (Plate 1, Figure 2), until finally the two ends meet. The groove around the now circular area becomes gradually deeper, the entire surface outside of the circular, polar area has become dark colored; the polar-area itself is composed of large white masses with dark outlines. In this way is formed the "vitelline plug" of Ecker. Plate 1, Figure 3. In a side view of an egg a few hours after the formation of the vitelline plug, one sees that the latter has become raised up from the surface of the egg, giving the appearance of a small white mass resting on or protruding from a dark colored sphere. Plate 1, Figure 4. A front or polar view of the same egg at the same period is shown in Plate 1, Figure 3. The plug retains this prominent position but for a few hours and then begins to sink into the egg; as it does so, the adjoining parts of the egg close around it until there is a very small, circular pit or depression left in the centre of the area formerly occupied by the vitelline plug. While the plug is thus being withdrawn into the egg, there appears on nearly opposite sides of the contracting area occupied by the vitelline plug, the walls of the anal part of the medullary fold. Plate 1, Figure 5. This change has, of course, produced a corresponding change in the outline of the egg, between which and the vitelline membrane there is now quite a well marked space, but which is greatest at the lower pole. The medullary folds extend forward towards the opposite or anterior pole of the egg, quite rapidly, so that by the end of the fourth day after the beginning of the formation of the vitelline plug, a stage represented in Plate 1, Figure 2, the two folds have met at the head end. Plate 1, Figure 6. The cephalic portion of the medullary fold is much widest and thickest and the cephalic ends of the lateral wall of the medullary folds are more widely separated than the anal ends. The space enclosed by the medullary folds is marked through its

longitudinal axis with a slight groove or depression, the medullary groove. The areas lying within the medullary folds on either side of the medullary groove, are the medullary plates, and in some instances, are composed of cells slightly larger and a trifle lighter colored than those of the remainder of the embryo.

The egg has meanwhile been changing shape, not only on the dorsal side, that marked by the medullary folds, but also at the anal end, in such a way that in a profile view of the latter region there is seen a depression or a sinuosity in the outline, showing that the originally spherical ovum is beginning to take on the elongated form of the embryo. Plate 2, Figure 7.

The medullary folds having become continuous, the process of folding in and uniting with each other to form the closed, neural tube advances with great rapidity; the entire process occupying eight or nine hours. The first well-marked change in the folds, after they have become continuous at the cephalic end, takes place at points in the lateral-walls about midway between the cephalic and anal ends, where they grow inwards towards each other, Plate 2, Figure 7; then the large, thick walls of the cephalic end rapidly grow towards one another and unite over the middle line of the medullary groove. Near the anterior ends, the cephalic portion of the folds meet and unite first, the union gradually extending backwards along the median line. At the extreme anterior end of the medullary folds however, a considerable space is left which is the last to remain unclosed. In this way a fusiform space, the *sinus rhomboidalis* comes to be left between the anal end and a point about midway between the anal and cephalic ends, where the folds first grew towards each other. Plate 2, Figure 8. This fusiform space, though, is soon closed over by the advancing folds, and is quickly followed by the closing over of the space left at the cephalic end. At the extreme anal end, the folds remain separate over a small area, the space formerly occupied by the vitelline plug, and form a rounded edge about this small cavity or pit. It becomes a definitely rounded cavity by the time that the first constriction, indicating the throat, is seen. While the neural tube has been thus rapidly forming, the embryo has increased very much in size, and its outline has become very much altered. It is now much more elongated, and both the anal or caudal and cephalic ends are becoming more definitely indicated as they grow away or stretch out from the body of the embryo. The entire

surface of the body is now covered with cilia, by aid of which it keeps up a horizontal rotatory motion upon its axis.

In a ventral view of an embryo, at about this stage, we would also notice this change in form, and we would see that the anal end of the medullary folds extend farther around on the ventral side than the cephalic end. Plate 2, Figure 9.

A constriction now makes its appearance in the throat region, thus defining the head from the body. At the same time, the remainder of the region of the neural canal becomes more distinctly outlined; a swelling or slightly oval prominence appears on each side of the head, the first external indications of the optic vesicles. Plate 2, Figure 10. In a dorsal view, a line running along the centre of the neural canal indicates the line of union of the medullary folds. Plate 2, Figure 11. In a ventral view of the same are seen both the optic vesicles, the ridge of the medullary fold between them, the constriction of the neck and the anus at the posterior end of the neural tube. Plate 2, Figure 12. The embryo having reached this stage, a second groove or furrow appears in the neck-region, so that the throat is now marked off both from the head and from the body. The anterior end of the neural canal or head now bends forward and downward upon itself, so that, by this cranial flexure, the fore-brain, with its optic vesicles, no longer occupies the anterior end of the longitudinal axis. The head has also changed in shape, having no longer a simple rounded outline. In the anterior portion of the neural canal there appear a few transverse swellings, the first indications of the protovertebræ. Plate 2, Figure 13. These latter soon increase in number, additional ones making their appearance posteriorly; the neck region becomes larger; the optic vesicles become more rounded and more prominent. There is next seen projecting from the sides of the neck behind and above the prominence of the optic vesicles, a pair of lobes, one on each side; from these lobes are to be developed the branchiæ. A little posterior to the branchial lobes, there has also appeared another pair of lobes; from these will be developed the anterior limbs. The optic vesicles are still more prominent, and the protovertebræ now appear in a side view to be somewhat removed from the outer edge of the neural canal towards its centre; they are also larger.

Development now progresses at both extremities, and the entire body increases rapidly in size. The head is still farther separated



from the body by the continued growth of the neck region; the branchial and brachial lobes are growing more prominent, and on the median ventral line of the neck between the branchial lobes, or slightly posterior to them, is a single rounded prominence which indicates the pericardial region. The posterior end of the body, owing to the development of the tail, which is stretching away from the body, has become more elongated, and is obtusely pointed.

In a ventral view at this stage, the nasal pits are distinctly seen, as two small, black cavities lying just within or ventral to, the swellings of the optic vesicles. The head is seen to have become much narrower and longer, and the position of the future mouth is indicated by the space existing between the anterior end of the branchial lobes and the curved outline of the extremity of the neural canal. The beginning of the tail also shows distinctly, and its median ridge, at the end of which is the dark cavity of the anus, is now much increased in size. Plate 2, Figure 14. At a period about two days later than that represented by Figure 14, a new lobe or prominence is seen upon each side of the neck between the eye and the branchial lobe; it is much smaller than, and lies just at the anterior extremity of, the long axis of the branchial lobe. Very often it is developed consentaneously with the branchial lobes, instead of making its appearance a day or two later. From these lobes are to be developed structures which, from their resemblance to the balancers of Dipterous insects, have come to be known as the "balancers." The eyes have progressed rapidly during the last day or two, and the nasal pits are more clearly defined. The body of the embryo is now, by a rapid growth of the ventral side, losing the curved outline which it has always had, owing partly to its having been formed upon a sphere, and is now becoming straight; the caudal portion is developing rapidly and vertebræ will soon be seen making their appearance within its substance.

The animal now begins to show active, muscular movements, which consist of a sudden doubling upon itself; a position retained for a few seconds only, when it regains its original position by another sudden and violent movement of the body. A thickened ridge also appears on either side of the anus; these are the walls of the cloaca. Within a day or two, the rapidity of development varying widely in different specimens, the branchial lobes show

traces of division into three portions; the divisions making their appearance first upon the ventral side and running at right angles to the long axis of the lobes. In this way the three pairs of gills are first indicated, and the divisions between the lobes are the first external indications of the branchial clefts. The small rounded lobes anterior to the gills have already become elongated and somewhat resemble their perfect form.

The integument over the pericardial region has become so transparent that the heart can be seen by transmitted light to be pulsating. Up to this period the embryos, since the closing of the medullary folds, have been of a uniform dark brown or brownish-black. Now, a number of large stellate cells filled with black pigment make their appearance along the region of the protovertebræ, from the branchial lobes nearly to the anus; others soon make their appearance in the same region filled with a greenish-yellow pigment and some of the external epithelial cells have the same yellowish-green hue. These pigment cells are very early found upon the brachial lobes and soon extend over all parts of the body. The body of the embryo is now straight and five or six vertebræ have been formed in its rapidly developing tail. Plate 2, Figure 15.

A dorsal view at this stage, or a little earlier than this, before the divisions appear in the branchial lobes, shows the body of the embryo resting on the unabsorbed yolk, of which there is still considerable left. It also shows very well the relative position of the eyes, balancers, branchial and brachial lobes, and the division between the neck and body. This latter differentiation is now becoming more and more evident. A ventral view shows that a deep constriction has taken place on the sides of the neck, thus marking off that region from the rest of the body. In the anterior end of the body region, where it has been made narrow by the lateral constriction, is the pericardial region; the integument is here so thin that the chambers of the heart may readily be distinguished and the pulsations counted.

The divisions of the branchial-lobes, or the branchiæ, as we may now call them, for the blood is by this time circulating in them, and the balancers all grow rapidly in length. The caudal portion of the body also becomes longer, but otherwise there are but few external changes posteriorly, for a day or two. Most of the energy seems to be devoted to the growth of the branchiæ and the balancers. In examining a large number of specimens, it is

at once seen that there is great variation in the progress of development. The position of the balancers too, varies considerably in different individuals of the same age.

Active growth is next shown in the development of the tail and the caudal and dorsal fin; the branchiæ and supporters are also growing rapidly, and a depression on the ventral side, on a line between the eyes, marks the position where the mouth will appear. The heart may still be seen in the pericardial region, though the integument is gradually becoming more opaque. It is now making from forty-eight to fifty pulsations per minute. Plate 3, Figure 16. During the following thirty-six hours, the branchiæ continue to progress rapidly, becoming more and more elongated, and begin now to bud out small processes from the sides. The eye has become much more perfect, and its structure is nearly complete. The balancers have grown with the gills, though they do not equal the latter in length. The caudal fin has become so large that it now performs its functions as the locomotor organ of the body. The animal shows quite active energetic movements in the egg, and if it is allowed to escape into the water by tearing open the membranous shell, it is seen to swim about with great activity, being propelled by vigorous movements of its tail. Watching its movements as it sinks to the bottom of the dish, which is covered with a deposit of fine, light, vegetable débris, we can readily determine the use of the balancers. As the animal approaches the bottom it holds its balancers out from the body so that they point outwards and downwards; owing to this position in which they are held, the animal sinks but a short distance into the light material of the bottom and thus keeps the head and branchiæ above the dirt where they can be readily furnished with a constant supply of pure water. The pericardial region is at the same time kept free from the bottom, so that there is nothing to interfere with the beating of the heart. Plate 3, Figure 17. This arrangement calls to mind the position which the cuttle-fish, *Loligo*, assumes when at rest; the tail and posterior portion of the body rest directly upon the bottom while the anterior portion is supported entirely by the median ventral pair of arms, only the anterior or distal ends of which furnish a support for the anterior portion of the body; the rest of the arms are arched so that the head and neck are kept from touching the bottom; thus affording free opportunity for the egress and ingress of water to and from the mantle-cavity and free use of the siphon.

A ventral view at this stage shows that the pericardial region is moved slightly further back, the neck region is not so narrow and the neck groove is continuous across the ventral surface. The outline of the mouth is indicated; the gill processes are increasing in size and in number; the balancers are still growing and have become somewhat capitate and the brachial lobes are beginning to increase in size. The head too is now changing shape, becoming much broader.

It is interesting and suggestive to note in a ventral view at this period, the general resemblance to a young dog-fish, especially in the position of the mouth and branchiæ and the shape of the head and body.

For the next two or three days development is most active in the branchiæ and in the tail. The latter increases considerably in length and the dorsal fin grows rapidly. The branchiæ double their length in two or three days and give off numerous processes which grow rapidly and which are arranged in two rows, the members of which point outwards and downwards, diverging from each other. The brachial lobes are developing slowly, being as yet, a pair of simple lobes or processes on the sides of the body just behind the branchiæ and partly covered by the latter. The change in the form of the head continues; it is becoming more rounded in front and broader. From this time until the posterior pair of limbs are being developed there is very little change externally, in the posterior portion of the body. The branchiæ and supporters have now reached their full development; that is, the branchiæ have all their processes budded out and the branchiæ are relatively to the size of the body as large as they ever will be, though absolutely they will still increase in size; the balancers, however, being only embryonic appendages, have attained their largest size; they are capitate and will now decrease in size and disappear as the anterior limbs develop and take upon themselves the function, previously performed by the balancers. Plate 3, Figure 18. After the branchiæ have become as large as those represented in Figure 18, the development of the anterior limbs may be best studied by cutting away the hinder pair of branchiæ. The limb-processes rapidly elongate, pointing backwards and a little downwards and outwards; at first, they are simple rounded processes with an unbroken outline until the length is two or three times the breadth. When they have attained these dimensions a slight indentation is

seen in the distal or free end of the limb, dividing it into two lobes each of which becomes a digit; the outer one, when the limb is directed backward, becoming the first or most anterior digit and the inner one becoming the second. A slight flexure or bend in the limb now makes its appearance which indicates the position of the elbow-joint. The opening of the mouth makes its appearance usually at about this stage or later. Plate 3, Figure 19. Soon after the first two digits are thus marked out, the balancers begin to diminish in size, becoming more and more slender but not decreasing in length. Plate 4, Figure 21. The mouth-groove is now fully indicated, but the opening appears first in the central portion of the groove and extends gradually in both directions, until the mouth has attained its full size. A side view shows that the tail has become longer, the dorsal and ventral fin-like areas have grown rapidly and the rectum is distinctly seen opening into the cloaca; the position of the mouth too has changed, being much farther forward. This condition is reached from the twenty-fourth to the twenty-sixth day after the formation of the vitelline plug.

The anterior limbs continue to grow rapidly; the second digit growing faster and quickly becoming much larger than the first and at the base of the second digit on the inner side of the foot appears a small process which is to develop into the third digit. Plate 4, Figure 24. The balancers are still more slender, the blood has nearly stopped circulating in them and they are of but little use. A central artery and vein are seen in the balancers when they first bud out from the side of the head, and these increase in length with the growth of the balancers; so that when the latter are fully developed the blood may be seen rapidly circulating throughout the length of these appendages; as they grow more and more slender there is less and less blood sent to them, until when they are in the condition represented in Figure 21, Plate 4, there are only a few stray corpuscles to be seen, which slowly work their way in single file to the extremity of the appendage and passing through the capillaries, as slowly wend their way back again. Circulation in the balancers now soon ceases and being of no further use to the animal, these appendages are no longer retained. While watching through the microscope, a specimen which had but one balancer left, and that a very slender one without any blood circulating it, I noticed that the creature would occasionally give a number of quick, violent shakes with its head;

as these were repeated I saw the balancer gradually break off at its base or proximal end and finally becoming entirely free, fall to the bottom of the dish, leaving the animal free of these embryonal appendages, for which it had no farther use. Plate 4, Figure 22. This observation was made upon a specimen twenty-eight days after the formation of the vitelline plug. In examining twenty-five specimens of this same age I found two in which both balancers were still present; three in which one still remained, and twenty in which both had disappeared. In all of these specimens development had progressed to the condition indicated by the presence of the rudiment of the third digit on the anterior limbs. Consentaneous usually, though sometimes a little later than the appearance of the third digit on the anterior limbs, appear a pair of small lobes on either side of the cloaca which are to develop into the posterior limbs. The progress of development in these appendages is like that of the anterior ones. The processes elongate, a slight indentation in the centre of the distal end appears, which increasing in size as the limb grows, forms two digits, the first and second; from near the base of the second, a process buds out which develops into the third digit; from near the base of the third digit buds out the fourth, and from near the base of the fourth buds out the fifth digit of the posterior limbs. The first indication of the first two digits of the posterior limbs occurs at about the same time that the fourth and last digit of the anterior limbs appear. Plate 4, Figures 23 to 28. All the external parts of the animal being now formed, the creature being about sixty days old, it undergoes no external changes beyond a general growth until the branchiæ begin to decrease in size as they are being resorbed. Plate 4, Figure 29. This change takes place in specimens reared in aquaria at about one hundred days from the beginning of segmentation. The process of resorption of the branchiæ begins at their distal ends; the outer branchial-processes become shorter and disappear, the outer portion of the main body of the branchiæ become shorter; then the inner processes disappear and nothing is left but three pairs of small rounded processes which are slowly absorbed; it taking as long usually for this latter part of the process to take place as it does for all the first portion. The whole process occupies from three to five days. Thus in a few days they change from water to air-breathers, from a less to a more highly specialized organization, and leaving the water take up their abode in damp localities upon the land.

To recapitulate briefly. After segmentation there appears around the lower pole of the egg an area made up of large cells, which, at first hemispherical, then oval and finally circular, forms the vitelline plug of Ecker. This plug protrudes from the egg, then sinks into it, while from the diminishing area around the disappearing plug, stretches away the anal portions of the medullary folds with the medullary groove midway between them. The two folds grow forwards and unite near the opposite pole. The medullary folds close in and unite forming the neural tube. The body elongates; is covered with cilia and rotates horizontally upon its axis. The head is marked off and the optic vesicles appear. The branchial lobes and the lobes of the cephalic-balancers appear; soon followed by those of the anterior limbs. The pericardial region is marked off and the pulsations of the heart are visible. The nasal pits and the position of the mouth are indicated. The tail and the dorsal-fin grow rapidly and the branchial lobes are divided into three pairs of branchiæ. The branchiæ give off processes, the eyes develop rapidly and the mouth is moving forward. The constriction takes place across the ventral surface of the neck, and the balancers now fully developed become capitate. The branchiæ become fully developed; the balancers become more and more slender as the anterior limbs increase in length, and the blood having ceased to circulate in the balancers they drop off. The anterior limbs now develop with rapidity, the first and second digits being formed first, then the third, and finally the fourth. The first two digits on the posterior limbs are formed as the fourth digit on the anterior limbs is budding out; then the third, fourth and fifth digits are developed in succession. About the one hundredth day after segmentation has begun, the branchiæ are resorbed and the animal enters the adult state.

Such was the case at least in those individuals which, having the most perfect branchiæ and the greatest amount of food, grew and developed most rapidly. Other specimens, however, which were surrounded by less favorable conditions developed more slowly. One which was hatched from the egg about the middle of May, retained its branchiæ until the last week in the following October, over six months, when, as the branchiæ were being resorbed, the animal suddenly disappeared from my aquarium during the night. From the time when the young are hatched to the period of the changing from the branchiate to

the abbranchiate condition, the dorsal and lateral surfaces of the animal are of a greenish-yellow hue appearing lighter or darker according to the amount of black pigment existing in the different specimens. In this respect there is considerable variation, though none of the specimens are very dark. In most of them yellow is more dominant than the green. The under surface up to and during the time when the branchiæ are resorbed is white with perhaps a slight tinge of yellow. In giving the course of development nothing has been said of the time when the embryo escapes from the egg; this was done because the time varies so very much. It occurs about the period that the balancers have reached their greatest size; sometimes however when they are only half-developed and again not until after they have begun to grow smaller.

The rate of development seems to be dependent upon a number of conditions. Some of the bunches of eggs are much larger than others, and while all those eggs in a small bunch of ten or fifteen will develop with very nearly equal rapidity, of the various individuals in a bunch of one hundred and fifty or two hundred some may progress twice as fast as others. Those upon the outside of the large bunches advance most rapidly and those nearest the centre the slowest. Temperature also has a marked effect; if the water is too cold it retards them, if too warm it kills them. The purity of the water too has an important influence; some which were supplied with running water growing and developing much more rapidly than others which were in jars where the water was changed but once or twice a day. While in the egg there is but little trouble in keeping them in good condition, but after they have escaped from the eggshells and have absorbed all the yolk-nourishment, I found great trouble in getting them food. I supplied them with various things but did not succeed in pleasing them. Three or four which were placed in an old aquarium where there were a number of snails and a good supply of Protozoa and vegetable growth, grew quite rapidly and did well, while those in my other aquaria developed cannibalistic tendencies, which were shown by their biting each others gills off and the tips of the tails also. A few only escaped mutilation in this way and these few increased in size much more rapidly than their less fortunate brethren. This rapidity of growth appeared to be of great benefit to them, for as soon as their mouths had attained the requisite size they turned upon the smaller members of their family and swallowed them



bodily. This large supply of food-material enabled these larger individuals to increase still more rapidly so that in two weeks from the time they commenced feeding upon their comrades they were ten times the size of one of the smaller ones of the same age, yet undevoured. Thus there was an interesting case of natural selection by survival of the fittest, going on amongst these young forms. Those who by their activity and strength preserved their branchiæ uninjured, develop so much faster than their brethren, as to enable them to pass through all their changes in the water and leave that element to seek for regions where food was more abundant. The power of reproduction of lost parts by this class of animals is so well-known that it seems remarkable that these young forms should not have reproduced their lost and mutilated branchiæ. But, on the contrary, not a single specimen of the many hundreds who suffered such losses, succeeded in restoring the lost parts. This may have been due to the small amount of food with which they were at that time supplied.

The branchial clefts have not been mentioned for the reason that they do not appear until after the branchiæ have become so large as to cover up the places where the clefts and arches make their appearance. It thus being impracticable to satisfactorily decide this point from external observations, it is left for the present and will be solved when I work up the changes in internal structure. For this work upon the internal parts I have preserved a large series of specimens in the various stages of development from which it is hoped, by means of sections, to get quite a complete history of the changes which there take place.

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### EXPLANATION OF THE PLATES.

The outlines of all the figures were obtained with the aid of the camera lucida.

#### PLATE 1.

Figures 1 to 5 are enlarged 18 diameters.

Figure 6 is enlarged 21 diameters.

FIGURE 1.—The lower side of an egg which has just completed segmentation; *v. p.* the area of large, light colored cells that are to form the vitelline plug; *v. p. f.* the begin-

**FIGURE 1—Continued.**

ning of the furrow around the plug. As yet the furrow extends not more than half way around the large cell area.

**FIGURE 2.**—A view of the same side of an egg, a few hours later; the letters as before. The furrow nearly surrounds the large cell area, and the latter is changing shape.

**FIGURE 3.**—Shows the same side of an egg, in which the fold has completely surrounded the area of large, light colored cells. This area is now circular, and is the vitelline plug of Ecker. Letters as before.

**FIGURE 4.**—A side view of Figure 3. The egg has contracted, leaving a considerable space between it and the vitelline membrane. This space is greatest and quite irregular in the region of the plug; the latter projects from the surface of the egg; *v. m.* the vitelline membrane. Other letters as before.

**FIGURE 5.**—A later view of the anal region; *v. p.* the vitelline plug which has nearly disappeared within the egg; *m. f.* the anal portion of the medullary folds stretching away from the area of the vanishing plug; *v. m.* the vitelline membrane; *m. g.* the beginning of the medullary groove.

**FIGURE 6.**—The dorsal region at a more advanced stage; *v. m.* vitelline membrane; *m. g.* the medullary groove; *m. p.* the medullary plate of one side; *m. f. a.* the anal portion of the medullary fold, and *m. f. c.* the cephalic portion.

## PLATE 2.

Figures 7 and 8 are enlarged 30 diameters.

The rest, 9 to 15, are enlarged 12 diameters.

**FIGURE 7.**—A dorsal view. Letters as before. The embryo has lost the circular outline of the egg and is changing shape rapidly; the medullary folds have assumed an irregular outline, and the point at which they will first unite is already indicated.

**FIGURE 8.**—A dorsal view of the same specimen, taken two or three hours later. The embryo is rapidly elongating and the medullary folds have united along most of their length. The sinus rhomboidalis is now one of the

FIGURE 8—Continued.

most prominent features. *m. f. c.* cephalic portion of medullary folds; *m. p.* medullary plate; *m. g.* medullary groove.

FIGURE 9.—A ventral view, less magnified, of a more advanced stage. *c*, the cephalic end of the medullary tube; *a*, the anal end of same.

FIGURE 10.—Lateral view of same specimen from which figures 7 and 8 were taken. Figure 10 was made twelve hours after figure 8. *n. c.* neural canal; *e*, optic vesicle; *t*, throat region; *a*, anus.

FIGURE 11.—Dorso-lateral view of same specimen as figure 10. *m. f. l.* line of union of medullary folds; *m. f. a.* anal portion of medullary folds. Other letters as before.

FIGURE 12.—Ventral view of specimen from which figures 10 and 11 were made. *cl*, the swollen mass from which the caudal portion is mainly developed. Other letters as before.

FIGURE 13.—Lateral view at a later stage. *e*, optic vesicle; *mb*, mid-brain; *bn*, the lobe from which the branchiae are to be developed; *ba*, lobe from which the anterior limb develops; *pr*, the external indications of the proto-vertebrae; *t*, throat region. From the condition represented in Figure 12 to that of Figure 13 the change of outline, with the exception of the increased cranial flexure, has been slight. The energy has been used in developing special parts, rather than in general growth.

FIGURE 14.—A ventral view. The embryo has been growing rapidly in the last two or three days; is much elongated, and the different regions of the body are acquiring definite limits. Letters as in Figure 13. Unfortunately, there is no reference to the nasal pits in this figure; they are the small, dark, oval depressions lying between the neural tube and optic vesicles. Compare Figure 15.

FIGURE 15.—Lateral view of a specimen considerably more advanced. The entire figure of the adult is quite well outlined; *n. p.* the nasal pit of the right side; *e*, the developing eye; *b*, the rudiment of the balancer of the right side; *n*, the pericardial region, with heart partly showing through; *bn*, the branchial lobe, which is beginning to divide into the three portions from which the branchiae of this side will develop; *ba*, lobe which gives origin to the anterior limb; *vt*, vertebrae; *pb*, black pigment

FIGURE 15—*Continued.*

in connective tissue-like corpuscles, which appear first in the dorsal region; *py*, yellow pigment in small cells resembling ordinary epithelium cells; *df*, dorsal fin; *vf*, ventral fin.

## PLATE 3.

Figure 16 is enlarged 12 diameters.

Figures 17 to 20 are enlarged 10 diameters.

FIGURE 16.—A lateral view; *f*, depression in which mouth is formed; *bl*, balancer; *h*, pericardial region; *n. p.* nasal pit; *a. l.* abdomen; *v. f.* ventral fin; *d. f.* dorsal fin; *n. f.* and *n. t.* neural or spinal region. The most rapid centres of growth at this period are the tail, dorsal fin, branchiae and balancers.

FIGURE 17.—Ventral view a day or two later than that of Figure 16. The region between the nasal pits and the anterior end of the body has been imperfectly represented in the figure. It is simply rounded. The position of the mouth is distinctly indicated by the groove *m*; the throat is clearly marked off from the body by a suture or depression; the balancers are developing rapidly and have become capitate; the branchiae are much elongated and are budding out lateral processes; the lobes of the anterior limbs show signs of active growth once more; *a*, the anus. The heart can no longer be seen through the thickened integument.

FIGURE 18.—A dorsal view; *n. t.* external indication of outline of brain cavity; *ba*, lobe of anterior limb. The caudal region has much increased in length; the branchiae are longer and have acquired numerous processes of considerable length. The limb-lobes are also more elongated.

FIGURE 19.—Represents the anterior end only; *bal*, balancer, now completely developed; *bn*, branchiae; *b. s.* branchial stump, the gill having been cut away to show the anterior limb; *b. a.* the anterior limb. The latter is now much elongated, the elbow-joint is indicated and the first and second digits.

FIGURE 20.—The same as Figure 19, but with the branchiae not cut away.

PLATE 4.

Figures 21 to 28 are enlarged 12 diameters.

Figure 29 is enlarged 14 diameters.

Figure 30 is one-half natural size.

Figure 31 is enlarged 100 diameters.

FIGURE 21.—View of anterior end of body and head; *bl*, balancer; these appendages are now becoming more and more slender, and the circulation in them is diminishing; *bn. s.* the branchial stumps, the branchiae having been cut away; *d1* and *d2*, the first and second digit of the anterior limb; *d3*, the first rudiment of the third digit.

FIGURE 22.—Shows the anterior portion only; *be*, the balancer which has just been shaken off by the animal. The branchiae are now fully developed. The digits of the front limb are elongating.

FIGURE 23.—A part of the hinder portion of the body; *ce*, the cloaca; *pa*, the posterior appendage budding out.

FIGURE 24.—Distal portion of anterior appendage of same specimen; *d1*, *d2* and *d3*, the first, second and third digits.

FIGURE 25.—View of cloacal region a little later; *ce*, cloaca; the distal part of the posterior appendage is bifurcating, giving rise to the first and second digits, *d1* and *d2*.

FIGURE 26.—Anterior appendage of the same; *d1*, *d2* and *d3*, the first, second and third digits; *d4*, the rudiment of the fourth

FIGURE 27.—The anterior appendage at a later stage and turned in the opposite direction; *d1*, *d2*, *d3* and *d4* the first, second, third and fourth digits.

FIGURE 28.—The posterior limb of the same. Letters as before.

FIGURE 29.—A portion of one side of the head and neck; *bn*, the branchiae which are being resorbed. The appendages of the branchiae have already been resorbed, and these rounded stumps will disappear in the course of three or four days.

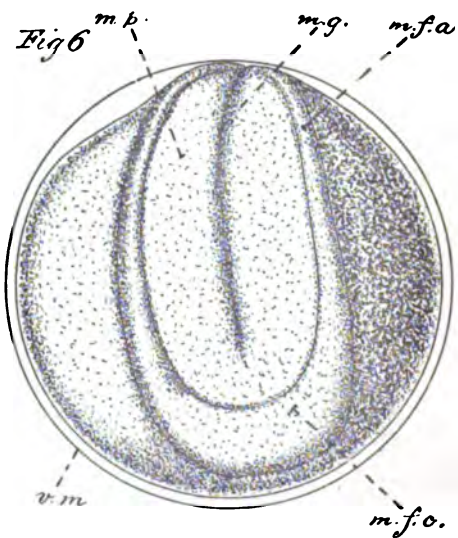
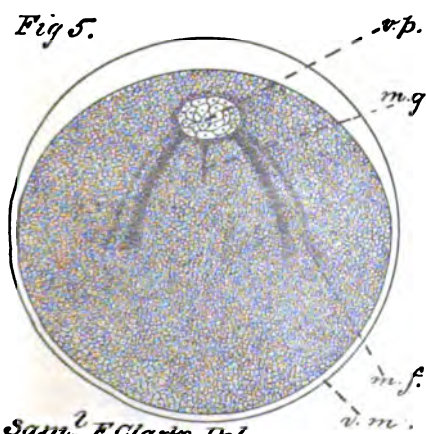
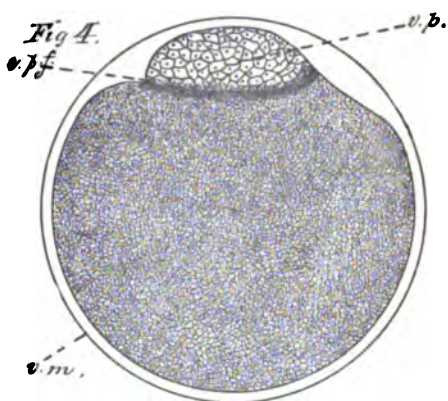
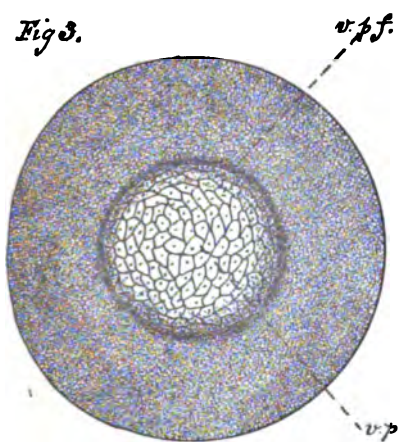
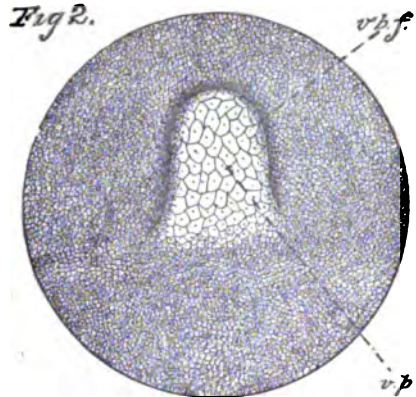
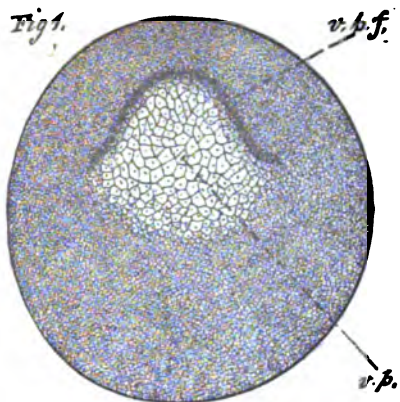
FIGURE 30.—Represents a bunch of eggs attached to a blade of grass. The double membranes about each egg show very plainly. The bunch from which this figure was made, contained over 100 eggs. It is one-half natural size.

FIGURE 31.—Four spermatozoa, enlarged 100 diameters only. *b*, has attached to it a remnant of the mother-cell; *c*, and *d*, have one end bent round so as to form a loop, which condition gives the appearance of the outline of a head.



*Development of Amblystoma.*

*Plate 1.*



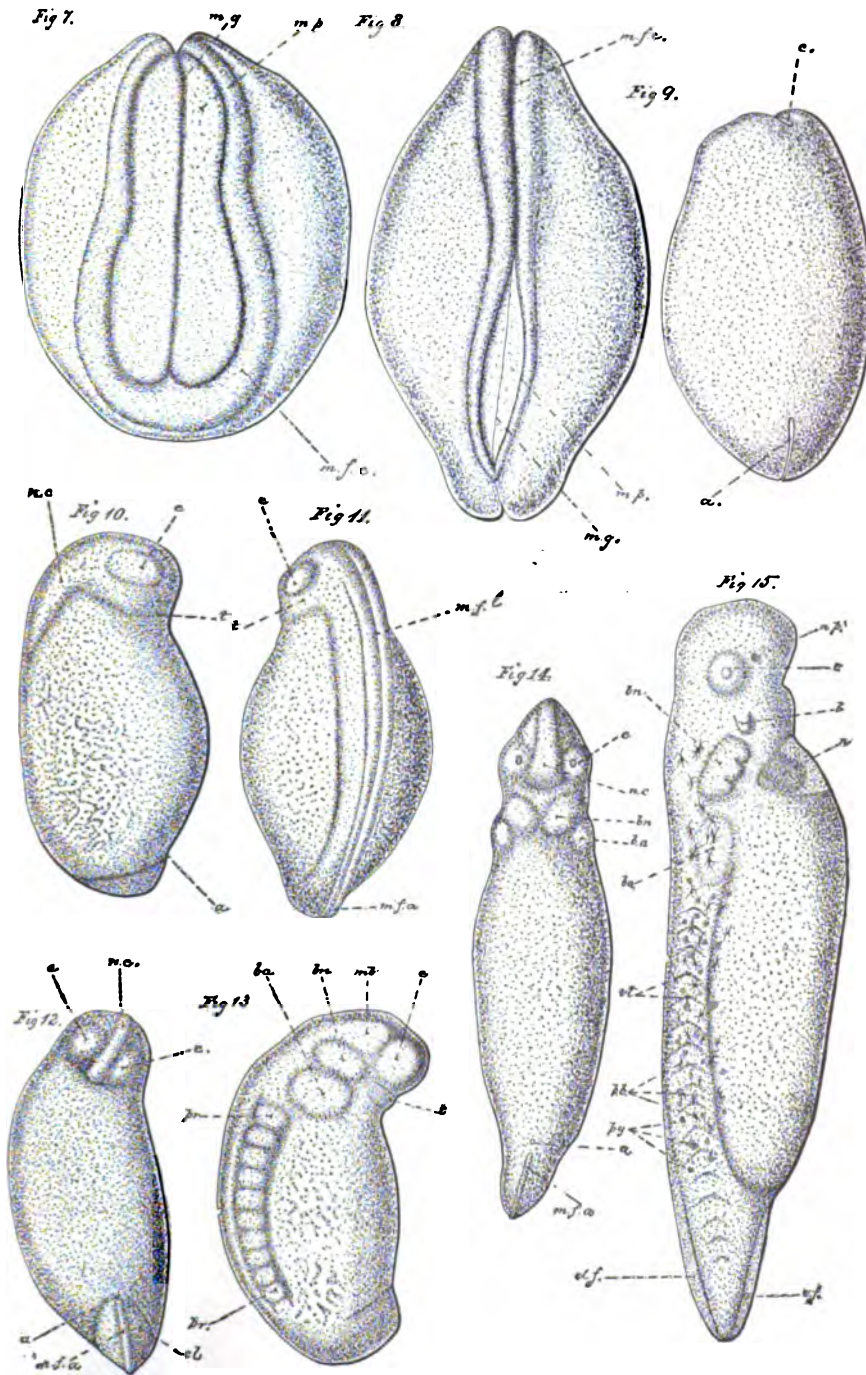
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**Development of Amblystoma.**

**Plate 2.**



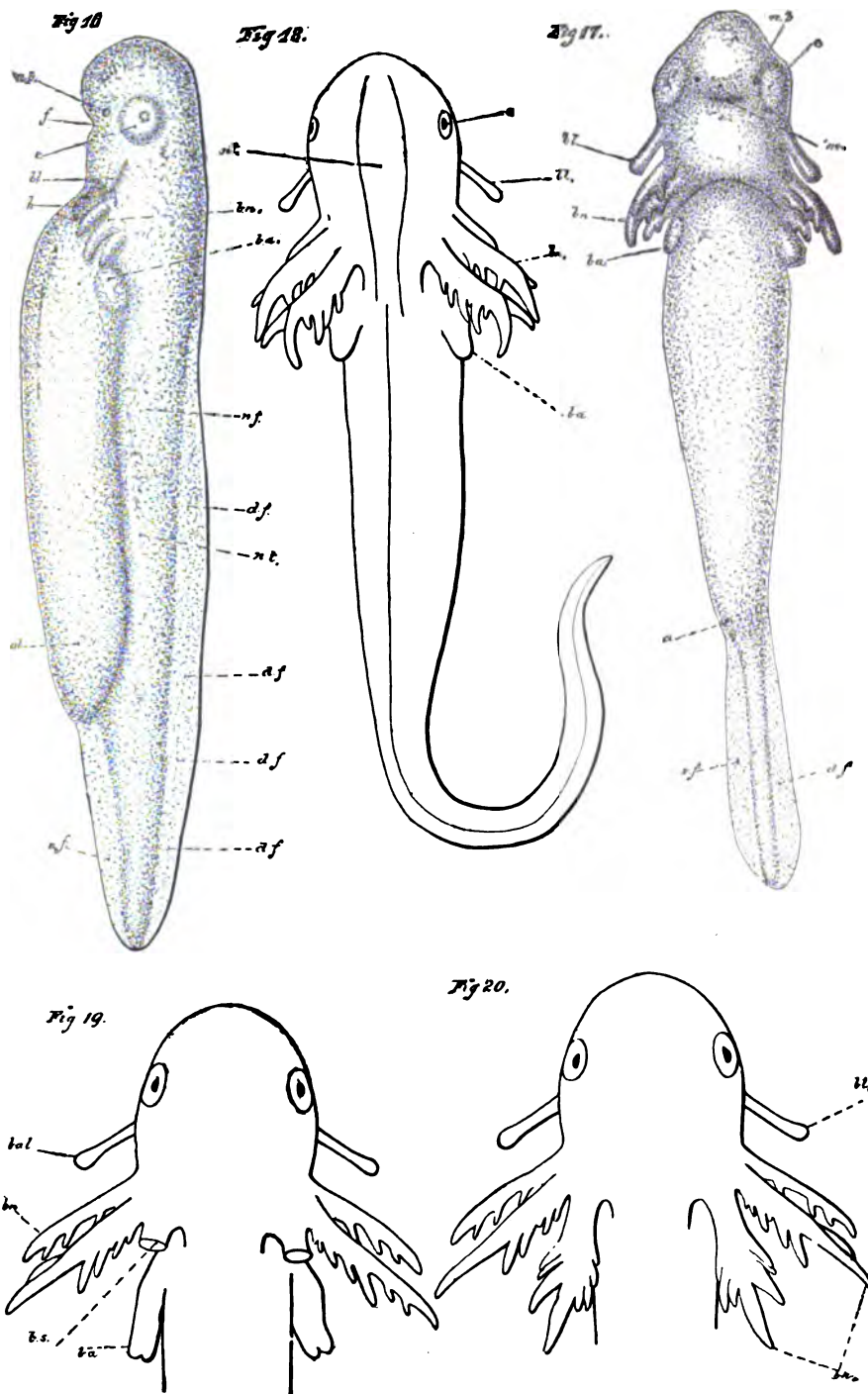
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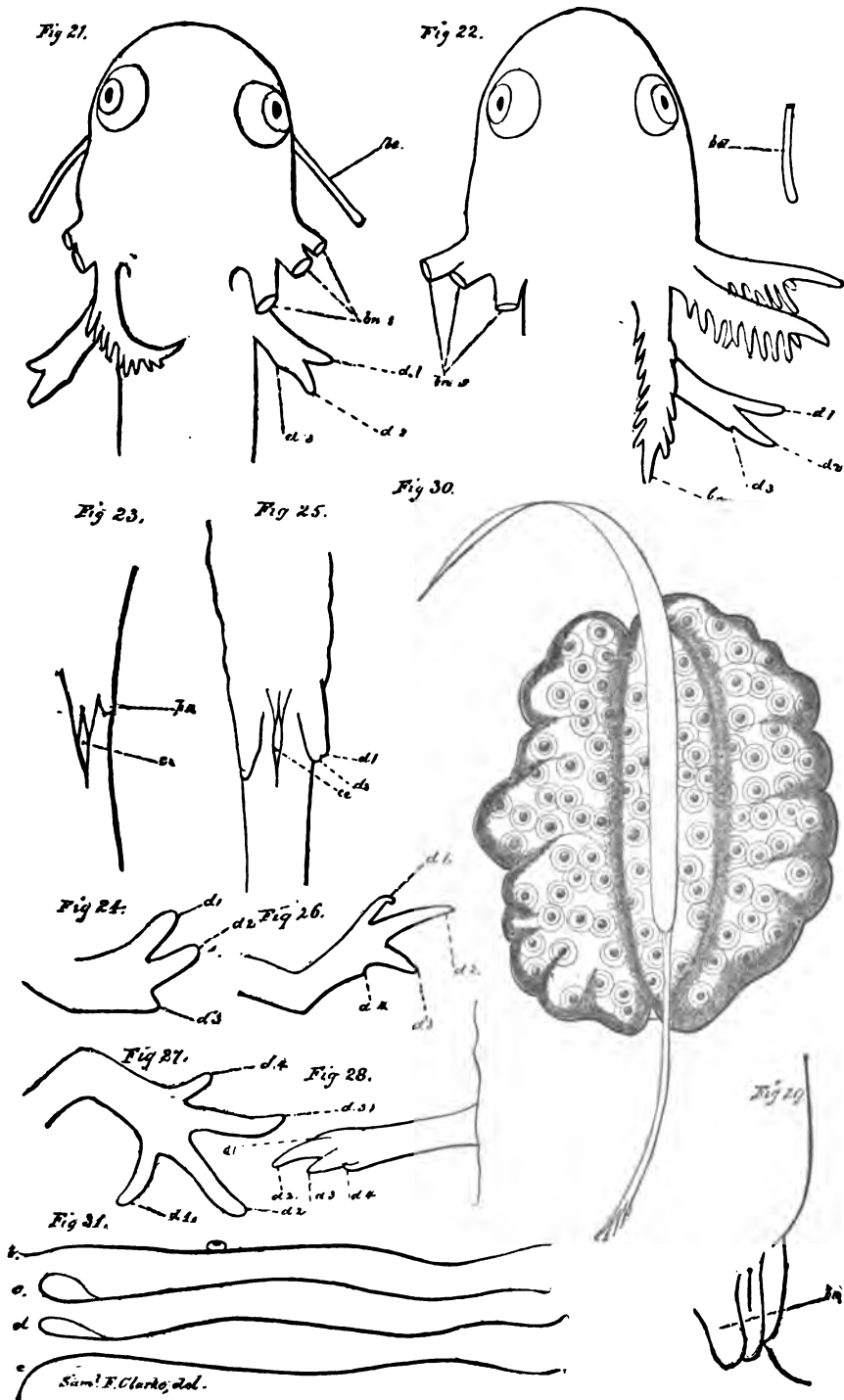
**Development of Amblystoma.**

**Plate 3.**



Sam'l F. Clarke, Del.







JOHNS HOPKINS UNIVERSITY,

BALTIMORE, MD.

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CHESAPEAKE  
ZOÖLOGICAL LABORATORY.

ORGANIZED AND CONDUCTED

By W. K. BROOKS.

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SCIENTIFIC RESULTS

OF THE

Session of 1878.

*(June 24th to August 19th, 1878.)*

LONDON: TRÜBNER & CO.

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While I have received most cordial responses from all to whom  
I have applied for help in the present undertaking, my thanks are \_\_\_\_\_

#### ERRATA AND CORRECTIONS.

The paper by Mr. Rice, on the "Early Stages of *Amphioxus*," has been delayed by the sickness of the author. It will be printed elsewhere at an early date.

On page 35, line 9 from top, *for* Mr. Crady, *read* McCrady.

" 36, " 10 " " " Mr. Crady, " McCrady.

" 85, bottom line, *for* Terebratulina, *read* Terebratula.

" 94, " " *for* ontogenetic, *read* phylogenetic.

" 95, line 4 from top, *for* ontogenetic, *read* phylogenetic.

" 112, " 17 " " " Mouth cavity, *read* Mantle-cavity.

" 113, " 1 " " " Typus, *read* typus.

" 116, " 6 " bottom, *for* J. R., *read* J. V.

" 116, " 4 " " " Regnaudii, *read* Reynaudii.

" 117, " 6 " top, *for* S. J., *read* S. I.

" 117, " 6 " bottom, *for* Heusen, *read* Hensen.

" 118, " 6 " " *for* Erforschung, *read* Erforschung.

" 119, " 2 " " *for* objection, *read* objective.

" 144, " 19 " top, *for* Plates 4 and 5, *read* Plates 12 and 13.

heretofore been regarded as within the province of an educational institution.

W. K. BROOKS, PH. D., *Associate in Biology*,

JOHNS HOPKINS UNIVERSITY,

BALTIMORE, MD.

Early Stages of *Amphioxus*. H. J. RICE. *Plates 14 and 15*, - 171

While I have received most cordial responses from all to whom I have applied for help in the present undertaking, my thanks are especially due

TO THE HONORABLE THE SECRETARY OF WAR, and to MAJ. GEN. Q. A. GILMORE, U. S. A., for the use of Government buildings at Fort Wool.

TO SPENCER F. BAIRD, U. S. FISH COMMISSIONER, and to T. B. FERGUSON, FISH COMMISSIONER OF MARYLAND, for valuable assistance.

TO S. M. SHOEMAKER, ENOCH PRATT, J. W. GARRETT, J. W. MCCOY, and other Citizens of Baltimore, for the funds to publish this Report.

TO THE TRUSTEES OF THE JOHNS HOPKINS UNIVERSITY for their liberal interest and assistance in a project which has not heretofore been regarded as within the province of an educational institution.

W. K. BROOKS, PH. D., *Associate in Biology*,  
JOHNS HOPKINS UNIVERSITY,  
BALTIMORE, MD.

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## INTRODUCTORY.

THE Trustees of the Johns Hopkins University having consented to the establishment, as a branch of the biological department of the University, of an experimental sea-side laboratory for the study of the marine zoölogy of the Chesapeake Bay, I visited the lower part of the bay in April, 1878, in order to select the best place for the experiment. Owing to the unhealthfulness of the shores of the bay, near the Capes, and the absence of suitable buildings, I was compelled to select a point at a considerable distance from the ocean, and the best point for the first attempt appeared to be the incomplected fortification known as Fort Wool. This fort is situated in the mouth of Hampton Roads, about one mile and a half from one shore, and three miles from the other, and about twenty miles from the ocean. It is an artificial island made by dropping granite rocks into the water, thus elevating an area of about six acres above high tide level. The area of six acres is almost covered by the incomplected fortification and building material, and the available space is thus reduced to about half an acre. In this space are two large frame

buildings, well lighted, near the water, and well adapted for use as a laboratory. Application was accordingly made, near the end of April, to Major Gen. Q. A. Gilmore, the officer in charge of the property, for permission to occupy them for the summer for that purpose. Through the kindness of the late Professor Henry and of Professor Baird, the Secretary of War was also informed in regard to the nature of the project, and on May 22d, the desired permission was received, and the following circular issued:

JOHNS HOPKINS UNIVERSITY.

CHESAPEAKE ZOÖLOGICAL LABORATORY.

A seaside laboratory for the study of marine zoölogy will be organized at Fort Wool, near Old Point Comfort, Va., during the present summer, as one of the departments of the Johns Hopkins University, and will be open for students on Tuesday, June 25th, 1878.

As the laboratory is especially designed to facilitate the researches of advanced scientific workers, no courses of lectures will be announced. There will, however, be an opportunity for a few less advanced workers, especially teachers, either ladies or gentlemen, to profit by the facilities which the laboratory will afford for collecting and observing marine animals, and such students will also receive proper help and guidance from those engaged in advanced work.

An efficient steward has agreed to superintend the table, and the price of board will not exceed five dollars (\$5.00) per week.

A fee of ten dollars (\$10.00) will be charged by the University for the use of the laboratory outfit during the season.

As the lodging rooms at the Fort are unfurnished, each student must provide all necessary bed-room furniture.

There will be daily communication during the summer with the Post Office at Old Point Comfort, and those who wish, will be able to lodge at the hotel.

The necessary outfit was then provided, and the laboratory was opened at Fort Wool, on Monday

the 24th of June, 1878, and was occupied until Monday, August 19th, or eight weeks. Owing to the unavoidable postponement of the announcement until so short a time before the opening, the number of applicants was quite small, and many persons who would have been glad to join the party were prevented by other plans which they had formed for the summer.

The following is a list of the scientific students of the party:

W. K. BROOKS, *Associate in Biology, Johns Hopkins University; in charge of the Laboratory.*

P. R. UHLER, *Associate in Biology, Johns Hopkins University.*

H. SEWALL, *Fellow, Johns Hopkins University*

CHR. SIHLER, M. D., *Fellow, Johns Hopkins University.*

H. J. RICE, " " " "

AUGUST SCHMIDT, *Teacher of Nat. Science, Zion School, Baltimore.*

N. B. WEBSTER, *Principal Webster Military Academy, Norfolk, Va.*

T. B. WEBSTER, M. D., " " " "

MRS. N. B. WEBSTER, " " " "

W. R. BOOKER, M. D., *Baltimore, Md.*

The laboratory was designed to accomplish four objects: to furnish advanced students with opportunities for original investigation; to provide material for winter work in the University; to enable less advanced students to become acquainted with the many forms of life which can only be studied at the sea-shore, and to give them an opportunity to become practically acquainted with the methods of marine zoölogical work; and to increase our scientific acquaintance with the zoölogy of the Chesapeake Bay.

The opportunities for advanced work in the study of invertebrate embryology were all that could be desired, and we had an unfailing supply of the most interesting material within our reach at all times. A strong current runs close to the walls of the fort, and thus carries a great body of water,—fifteen or twenty miles—past its walls at each turn of the tide, and free swimming animals and embryos and locomotive larvae could be obtained in endless variety without leaving the fort. The waters are especially rich in crustacean and anellidan larvae, and for work upon these groups at the station, it furnishes unrivalled advantages. Among the interesting forms of life which were most abundant, I may mention *Sagitta*, *Appendicularia*, *Torinaria* and *Balanoglossus*, *Pilidium*, *Actinotrocha*, and other important trochic larvae, *Squilla* and *Porcellana* in all stages of development, *Amphioxus* at all stages, and other forms of equal scientific importance. An abundant supply of material for work upon the adult structure and development of the Sponges, Hydroids, Medusae, Ctenophorae, Gephyreans, Anelids, Crustacea, Tunicata, Polyzoa, Brachiopods, Lamellibranchs, Gasteropods, and lower Vertebrates, is within easy reach. We found very few Echinoderms, and only one Cephalopod, but these groups could no doubt be found more abundantly by dredging nearer the mouth of the bay. We



collected near the fort, representatives of the Echini, Starfishes, Ophiourians and Holothurians, but not in abundance; and we found no Echinoderm larvae.

This list will show that there is ample opportunity for the less advanced student to become acquainted with marine life, and although no lectures were given, the students had free access to a very good collection of books on marine zoölogy and the proper reading to be done in connection with the study of each form of life was pointed out by the Associate in charge of the laboratory.

These students had the benefit of all distant excursions, and were thus enabled to become familiar with the methods of deep water and shore collecting, and to acquire familiarity with the habits of marine animals while gathering the material for more careful study in the laboratory. Here each student had ample room for dissection and for microscopic work, as well as the facilities for keeping and observing living animals in aquaria.

The following papers give an account of some of the scientific results of the first summer's work, and from them naturalists will be able to judge for themselves as to the advantages which the station offers for original investigation. As the main object of the laboratory is to furnish ad-

vanced workers with opportunities and facilities for carrying on their studies at the sea-shore, its future will depend upon the number of such persons who wish to make use of it. While it is to be hoped that there are at the present time enough persons engaged in morphological and embryological work in this country to make the want which the laboratory aims to meet a real one, this can only be ascertained by experiment. I have entitled the following papers the "Scientific Results" of the first session, but all who have been engaged in similar work know that only a very few of the subjects which are investigated can be carried sufficiently far in a single season to make their publication desirable. In addition to the subjects treated in the following papers, many other investigations were carried sufficiently far to yield interesting results, but their completion and publication must be delayed until favorable opportunities again present themselves.

A great part of our time was devoted to the collection and study of the microscopic embryos and larvae of the various forms of marine life, and as those who are not familiar with this department of zoölogy may be interested in hearing how animals too small to be examined without a microscope are collected in the open ocean, a short account of our methods will not be out of place

here. Nearly all marine animals discharge their eggs, in very great numbers, into the water, and the young are left to provide for themselves, and whether the adults are swimming animals or those which live at the bottom, or those whose proper habitat is along the shores and marshes or those which are fastened to one spot like the barnacle and the oyster, the young are, in nearly every case, fitted for swimming, and during this wandering period of their lives are diffused over a great extent of water by the tides and currents. They seldom bear any very close resemblance to their parents, and frequently undergo the most remarkable transformations before they reach the adult form. During the day and in rough weather they are scattered at a distance below the surface of the water, but on calm nights they come to the surface in inconceivable abundance, and may then be gathered by a method known as surface collecting, which is the very poetry of life at the sea-shore, although it is useless to attempt to describe the charm and fascination of midnight work in a small boat upon the calm surface of the ocean in the perfect quiet of an August night. About an hour after dark, on a summer evening, as the evening breeze dies away the water begins to grow phosphorescent, and the swell breaks upon the shore in lines of light. As the smaller animals accumulate at the surface the light grows more

brilliant and about ten o'clock, if the evening is a favorable one for surface collecting, the water, when disturbed by oar or net, is filled with star-like sparks, each one of which is an organism.

At the proper time a party of two push off into the darkness in a small boat, and guiding themselves by the light in the water, find a spot where an eddy or current has brought great numbers of animals together. The collecting apparatus consists of a bucket nearly filled with sea water, and a short handled net, about six inches in diameter and two inches deep, made of bolting cloth, or of a finely woven silk veil. As this net is gently moved through the water, the animals adhere to it, and its surface flashes out into a brilliant glow, as if it had been heated to redness. After two or three gentle dips it is raised and inverted over the bucket of water, and the sparks washed off, and the process repeated. After an hour or more of collecting, the bucket of water is carried home for examination. At the laboratory, a lighted lamp is placed in the centre of a table, and tall jars or ale glasses are filled with water from the bucket, and held up before the light for examination. If the collecting has been very good the water will be found to be crowded with small living animals so that the light can hardly penetrate it, and the bucket must be diluted with another of ordinary sea water before it can be examined. When this

is done each glass will be found to contain strange organisms in sufficient variety to furnish the material for weeks of study. As the water slowly circulates around the glass, carrying the various animals past the eye, it presents an indescribable shifting panorama of strange, grotesque and beautiful animals, most of them perfectly transparent, and varying in size from those too small to be seen without a lens, to those half an inch long. Nearly every glass contains small hydro-medusae expanding and contracting their transparent bells and slowly rising and sinking in the water; young Ctenophorae with iridescent zones of steel blue, and green and purple flashing over their bodies; the transparent embryos of fishes, their large black staring eyes and golden yolk sacks attracting the eye, although their shadow like bodies are scarcely visible. Numerous veliger embryos of various Mollusks are usually to be seen, expanding their transparent sails, and rising slowly to the surface by the beating of their iridescent cilia, and then darting back into their shells and sinking slowly to the bottom. Occasionally an Amphioxus embryo starts up from the bottom of the vessel, and after darting from side to side, stiffens and sinks again to the bottom, while an endless variety of grotesque crustacean embryos dart vigorously in all directions. Resisting the temptation to examine all the novel forms which attract his eye, each student

catches with a dipping tube the animals upon which he is working at the time, and places them in watch crystals, tumblers or small jars, with fresh sea water, in order to preserve them for his work.

As regards the number and variety of forms found by surface collecting, Fort Wool is superior to any other locality with which I am acquainted.

The accompanying papers give some of the scientific results of our work, but as they are written to be read by specialists, I will try to give a brief statement of some of the more interesting points, for the benefit of the unscientific friends who have aided in the organization and management of the laboratory and in the publication of the papers.

The lists of animals and plants found at Fort Wool, by Prof. Uhler and Prof. Webster, are by no means complete, but they are of great interest, as showing the rapidity with which land animals and plants gain access to an isolated spot. Although the fort is simply a pile of rocks, with only such soil as was made by the chips of the stone cutters, the list of land animals and plants which have gained a footing there is quite extensive. It is interesting to note also that although the period during which these forms have been isolated is quite short, less than twenty years, Prof. Uhler has found one case, the Cricket, in

which the form at Fort Wool is a well marked variation.

The paper by Mr. Rice, upon *Amphioxus*, is one of especial interest, as *Amphioxus* is the lowest representative of the group Vertebrate, which includes all the higher animals and man.

*Amphioxus* is a small worm-like animal, without a skeleton or hard parts, and with the brain almost wanting, and without eyes or other sense organs. It lives in the sand at the bottom of rather shallow water, and while it agrees with the higher vertebrates in so many points, that its relation to them is undoubted, it is very much simpler in organization than many of the invertebrates.

A thorough knowledge of every thing which relates to its structure, habits and development, is of great scientific value, as it is the key to a correct knowledge of the higher vertebrates; the generalized diagram or abstraction of the group Vertebrata as a whole. It has been very thoroughly studied by European zoölogists, and numerous papers upon it have been published within the last few years, and Mr. Rice has been able to supplement these observations, and to supply facts which render our acquaintance with it more complete. During the past year, *Amphioxus* has been found in the Bermudas, and it has also been found upon the coast of North

Carolina, but previous to the past summer these were the only places where it was known to exist upon this side of the Atlantic. Our discovery of it in the Chesapeake Bay, renders it accessible to any American Naturalists who may wish to study it.

Although there is no doubt that many of the lower animals which are found in the Chesapeake Bay have never been described by Naturalists, we made no attempt to discover and describe new species. This work can be thoroughly and satisfactorily done only at places where large collections and libraries are accessible, and it is more properly *museum* than *laboratory* work; while a sea-side laboratory furnishes opportunities for work of a different character, which cannot well be carried on in museums; the study of the structure, development and habits of animals as living things. We accordingly tried to study a few forms thoroughly, instead of searching for novelties. In our collecting, we met with many species which there is reason to believe are new, and these were preserved, and descriptions of such as prove to be new will be published after they have been properly studied.

During the latter part of the session, Mr. Schmidt, one of the students of the party, collected a few specimens of a small crustacean of the genus *Leucifer*, and as not only the species, but the genus



is new to American waters, I sent one of the specimens to Prof. Walter Faxon, of Harvard College, a specialist in that department, and he has prepared an illustrated description which forms part of this report.

I hope that my own principal contribution to the report, "The Development of Lingula," is not without scientific value. Lingula is a small animal which is found as a fossil in almost the earliest fossiliferous rocks, and has been in existence ever since, and is found in geological formations of all ages. It is a very remarkable exception to the general law that forms of life, like individuals, have a limited duration, which, geologically speaking, is quite short. As far as we have evidence it is the oldest animal now living, and almost the earliest which is found as a fossil. It is a small animal, composed of a fleshy cylindrical stem or root, about two inches long, and at the end of the stem a pair of horny tongue-shaped shells, between which the body is placed. It lives in the sand, in from one to ten fathoms of water, with its stem buried in the sand, and the tip of the shell projecting. The mouth, which is between the two shells, is surrounded by a crown of several hundred arms, which are covered by microscopic hairs or cilia. These cilia are in constant motion and cause currents in the water, and by these currents small plants are continually swept into the animal's

mouth. Its long duration is to be explained, perhaps, by the great simplicity of its conditions of life, for ever since the sedimentary rocks began to be formed, banks of sand must have existed in some part of the ocean. The horny shell of *Lingula* is quite tough, and easily preserved, and the animal lives in the proper situation for being readily preserved as a fossil. While there are undoubtedly other animals which have existed for as long a time we have no record of their existence, as their remains have not been preserved in the rocks.

It will readily be understood that a thorough knowledge of every thing relating to *Lingula* is of importance in the study of the laws according to which living things have been produced, and I regard the observations which I was able to make upon its development, as among the most important results of our summer's work.

W. K. BROOKS,

*Associate in Biology,*

*Johns Hopkins University.*

## Partial List of the Land Plants found at Fort Wool.

BY N. B. WEBSTER, *Norfolk, Virginia.*

THE flora of the six-acre pile of granite rocks, rendered dear to the memory of the members of the Chesapeake Zoölogical Laboratory by their pleasant summer residence there, is interesting, as showing what plants gain a home on almost soilless, barren rock and its detritus.

It is not probable that any of them were *purposely* introduced, but that seeds were carried by birds, wafted by winds or waves, conveyed in hay, straw, or packing materials, or incidentally scattered by soldiers or visitors who threw away peach kernels, &c., among the rocks. Not less than fifty species of plants grow luxuriantly on the island, many of which I had no opportunity to determine.

*Arenaria serpyllifolia.* Thyme-leaved sandwort.

*Aristida dichotoma.* Beard grass.

*Ambrosia artemisiæfolia.* Hog-weed.

*Baccharis halimifolia.* Sea-groundsel tree.

- Capsella bursa-pastoris.* Shepherd's purse.  
*Cyperus echinatus.* A common sedge.  
*Dactylis glomerata.* Orchard grass.  
*Ficus carica.* Common fig, growing under difficulties without much earth. Leaves tough and deeply incised. No fruit.  
*Fragaria vesca.* Strawberry.  
*Juniperus Virginiana.* Red cedar.  
*Linaria vulgaris.* Butter and eggs.  
*Melilotus alba.* Sweet clover.  
*Maruta cotula.* May-weed.  
*Opuntia vulgaris.* Prickly pear.  
*Oenothera biennis.* Evening primrose.  
*Phytolacca decandra.* Pokeberry.  
*Persica vulgaris.* Peach.  
*Portulacca oleracea.* Purslain.  
*Polypogon Mouspeliensis.* Polypog grass.  
*Poa pratensis.* June Spear-grass.  
*Rumex crispus.* Curled dock.  
*Rubus strigosus.* Red raspberry.  
*Rubus villosus.* Blackberry.
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## List of Animals observed at Fort Wool, Va.

BY P. R. UHLER.

A RESIDENCE of about one week, in August, at the Chesapeake Biological Laboratory enabled me to make a list of such creatures as occurred there and in the adjacent waters at that time. This list is of course very incomplete and omits whole groups of objects which might have been found there either earlier or later in the season.

Each year has its peculiar forms in such localities; and so a period of several years of close observation would be required to secure a tolerably full catalogue of the genera and species belonging to the region.

As this island is an artificial one, with only a slight layer of soil upon the surface of large blocks of stone, covering an area of less than six acres, but little can grow to sustain life, and every kind of creature found thereon has been brought from some other locality. Yet, many forms of life are indigenous to the surrounding waters, and a fine harvest of them may be gathered at most times upon and between the rocks forming the outer boundary.

The place is conspicuous for the absence of most things belonging to fresh water, and hence the Dragon flies and other insects dependant upon that element in their young stages must come from the not distant mainland.

The submerged rocks and adjacent waters are well stocked with Mollusks, Crustacea, Bryozoa, Hydroids, and various Sponges and Sea-Weeds, and around the wharf Fish of several kinds may readily be taken with the hook and line.

## MOLLUSCA.

### CYPRALOPITA.

#### *Cypralopita* TOKUY.

Occasionally captured by seines and trawl nets, and a few have been taken by accident from the stomachs of Sea-fish. *Cypralopita setacea* Linn.

### CASTRALOPITA.

#### *Castralopita* ALLEN.

This was first taken in a trawl net in a few days after the first of June.

#### *Castralopita* ALLEN.

This was first taken in a trawl net in a few days after the first of June. It lives in the water, and is found in the water, where the water is not so deep as the other has extended on

from the shores. On the Atlantic coast of Virginia and Maryland it is very common near and in the muddy inlets and around the islands.

*Ilyanassa obsoleta*, Stimpson.

Common in shallow places where the bottom is mixed sand and mud.

*Urosalpinx cinerea*, Stimpson.

Very common on the rocks below high water mark. It was very variable in size and in thickness of the shell. The larger specimens were often observed to be coated with sponge, cases of *Serpula*, barnacles and sea-weed, and this was particularly the case with dead specimens which stuck in crevices of the rocks.

*Eupleura caudata*, Adams.

Dredged in shallow water on sandy bottom, mixed with mud.

*Lunatia heros*, Adams.

Common in deeper water near Fortress Monroe, and obtained by dredging.

*Neverita duplicata*, Stimpson.

Very common on sandy bottoms. It grows to a very large size in this region, as it does also in many places near the entrance to Chesapeake Bay, and in the adjoining Ocean.

*Crepidula plana*, Say.

Found sticking to shells and among oyster rubbish on slightly muddy bottoms.

*Crepidula fornicata*, Say, (Lamarck?)

Quite common among oysters, and often found adhering to their shells. Met with in several places between the rocks on the outside of the Fort, below low water mark.

*Crucibulum striatum*, Adams.

Dredged in places where broken shells and rubbish abounded, in water about eight fathoms deep.

*Littorina irrorata*, Gray.

Extremely common on the rocks and in the ditches within the fortification. It abounds on the Eel-Grass and upon water plants of the fresh and salt marshes of both shores of Chesapeake Bay.

## CHITONIDAE.

*Chiton cinereus*, Linn.

Found upon the shell of an oyster dredged in ten fathoms depth. It is quite common on oysters and other shells along the Atlantic coast of Northampton county, Virginia. No reddish specimens have thus far been reported from the region of Virginia.



## NUDIBRANCHIATA.

*Doris bilamellata*, Linn.

Two specimens and eggs were found by Dr. Christian Sihler, in tide pools beneath the wharf. The identification of this species may not be strictly accurate; but from the appearance, size and form of the specimens they approach nearest to that here given.

## LAMELLIBRANCHIATA.

*Teredo navalis*, Linn.

Broken shells of this creature occur among the rubbish at the bottom of the water. It is common in the Elizabeth River, and will no doubt be found in the submerged parts of the piles which support the wharf.

*Petricola pholadiformis*, Lamarck.

Occurs in muddy places in shallow water. It is quite common in the inlets adjoining the mouth of Chesapeake Bay, and in company with a very small form of another genus may be found imbedded in the hard clay of the shallow oyster beds.

*Mya arenaria*, Linn.

In muddy sand along the margins of both shores of Virginia, also near Fort Monroe, and along

the Atlantic shores of the Eastern Shore of Maryland and Virginia. It is also found at the mouths of rivers on both sides of nearly the whole extent of Chesapeake Bay.

*Cochlodesma Leanum*, Couthoy.

Single valves occurred among the broken shells dredged from sandy and mixed bottoms.

*Ensatella Americana*, Verrill.

In the muddy sand along the shores of Virginia, Maryland, &c. Odd valves and fragments of this razor-shell were dredged a few rods from the Fort.

*Macoma fusca*, Adams.

Common in dark oyster mud in shallow water.

*Solen viridis*, Say.

A few young specimens were dredged at various distances from the wharf. They were generally from a muddy sandy bottom.

*Mulinia lateralis*, Gray.

Dredged at various points in water from five to ten fathoms in depth.

*Venus mercenaria*, Linn.

Quite abundant on muddy beds in shallow water and along both coasts of the Chesapeake, in Southern Maryland and Virginia.

A young clam with concentric elevated ridges was dredged in five to ten fathoms on sandy bottoms. It conforms pretty nearly with the figure of *Mercenaria fulgurans* Tryon, Amer.

Marine Conchol. pl. 27, No. 391, but is smaller, probably younger, and lacks the zigzag lines there given.

*Macra ovalis*, Gould.

In sandy places at moderate depths and on sand-bars. Young specimens brought up by the dredge at various points.

*Yoldia limatula*, Stimpson.

Small specimens mixed with the fragments of other shells, were brought up from pasty mud by the dredge. They are less than one-half the size of those found on the coast of New England, and may prove to be specifically distinct. A similar, if not identical form, lives well buried in the sticky mud of the Patapsco, and other rivers of our Western Shore of Maryland.

*Arca ponderosa*, Say.

Dredged from sandy bottoms, mixed with mud, in water eight to ten fathoms deep. It is moderately common in the Ocean, off the coast of Northampton County, Va., in holes fifteen to twenty fathoms in depth. At Assateague inlet, it may be dredged from the deeper water in the mouth of the channel, in company with the succeeding species.

*Arca Americana*, Gray.

Specimens of medium size occur in the same places as the preceding. Both of these forms

are covered with a hispid blackish coat, which is rarely found intact, and which gives them the appearance of being wrapped in woolen plush.

*Modiola plicatula*, Lamarck.

Found adhering to oysters, and wedged between rocks, particularly where mud has settled. It is distributed over most parts of Chesapeake Bay, south of Tangier Sound, and abounds in the adjoining Ocean wherever oyster beds have place.

*Modiola modiolus*, Turton.

Common between the rocks and on oyster bars in muddy places. Very abundant on the Atlantic coast of Maryland and Virginia.

*Anomia glabra*, Verrill.

Dredged in many places near the Fort, and some specimens were found attached to the rocks near and beneath the wharf; also on oysters.

*Ostrea Virginiana*, Lister.

Quite numerous attached to the rocks on the Western and Northern sides of the island. It is of the form common to York River, and vicinity of the mouth of James River. A few specimens may be selected which show the scalloped edges and ridged surface of the typical form of the Eastern side of Chesapeake Bay. A third form, resembling the round, small, deep shells of the Raritan River, inhabits the bars in the tidal part and mouth of the Patuxent River.

CRUSTACEA.

BRACHYURA.

*Ocypoda arenaria*, Say.

Not found upon the island, but may be collected on the sandy shore near Fort Monroe.

*Gelasimus pugilator*, Lat.

Common on the adjacent shores, particularly near Fort Monroe. At the latter place it occurs in countless numbers upon the muddy sand flats, in company with another species not determined.

*Pinnotheres ostreum*, Say.

Occasional in oysters.

*Panopaeus depressus*, Smith.

In muddy places and among the oysters in shallow water.

*Platyonichus ocellatus*, Lat.

Moderately common in the sand not remote from shore; and occasionally dug out by the dredge in shallow places.

*Neptunus hastatus*, Milne-Edw. (Say).

Very abundant near the wharf, and very generally distributed throughout Chesapeake Bay and along the Atlantic coast. It breeds and develops in this vicinity.

## ANOMOURA.

*Hippa talpoida*, Say.

Common in sandy places near the adjacent shores. The young ones may be found around the edges of the island.

*Eupagurus*, sp.?

Two forms of these Soldier Crabs inhabit shells of mollusks among the rocks and on the bottom around the island: but the species have not yet been determined.

## MACRURA.

*Charybdis calappa* Fab.

Occurs around the rocks near the wharf.

*Portunus calappa* Stimpson.

This is the common Soldier of Chesapeake Bay, and it may be found almost everywhere in shallow water.

## AMPHIPODA.

*Squilla*, sp.?

Common and seen and between the rocks near the wharf.

*Corophium*, sp.?

Common in the water near the wharf and among the *Sargassum* growing on the rocks: but

extremely abundant inside of the oscules of a roundish form of sponge common to that vicinity.

ISOPODA.

*Livoneca ovalis*, Harger.

Taken from the gills of a Croaker fish caught at the wharf.

CIRRIPEDIA.

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Adhering to the piles of the wharf in moderate numbers, and not uncommon on the rocks and on shells of Mollusks.

ANNELIDA.

*Nereis*, sp.?

Common in muddy places and upon oysters.

Other Annelids occur in the water around the Fort, but the species have not yet been determined.

RADIATA.

One or more *Holothurians*, an *Asterias* and an *Ophiopholis* may be dredged from the water in the near vicinity; but their names have not yet been determined.

As I am in doubt as to the names of the *Bryozoa*, *Acalephs* and *Hydroids* common to the rocks and waters around the island, they must be left unrecorded at present.

#### SPONGOZOA.

*Microciona prolifera*, Verrill.

Quite common on oysters, and also to be found adhering to rocks beneath the wharf.

This species of Sponge harbors *Paramecium*; and by placing a piece of it in a vessel of water, countless swarms of these little creatures may be seen streaming forth in all directions.

#### INSECTA.

##### LEPIDOPTERA.

*Vanessa (Grapta) interrogationis*, Fab.

One specimen seen flying around the wooden barracks.

*Papilio troilus*, Linn.

Three specimens were observed flying over the flowers of *Miliola*.



*Papilio asterias*, Fab.

One specimen with damaged wings seen flying about the tall weeds.

*Danais archippus*, Fab.

Several specimens seen flying vigorously over the island, and occasionally alighting upon the taller weeds.

*Utetheisa bella*, Pack.

Two or three specimens were aroused in the thickest patches of weeds.

Three or four species of small Moths were seen; but they were too imperfect for identification.

## COLEOPTERA.

*Cicindela punctulata*, Fab.

Quite common in the paths, and all the specimens noticed were of the dull black variety.

*Pangus caliginosus*, Fab.

Only two specimens seen. They were crawling about in the midst of the weeds and rubbish.

*Harpalus bicolor*, Fab.

Common beneath loose stones and rubbish.

*Stenolophus plebeius*, Dej.

Found beneath loose stones and rubbish; not common.

*Cercyon prætextatum*, Say.

Seen pushing its way among loose rubbish, next the ground.

*Collops quadrimaculatus*, Fab.

On various bushes and weeds; quite common.

*Diabrotica 12-punctata*, Oliv.

Common on *Baccharis* and other bushes.

#### HYMENOPTERA.

*Apis mellifica*, Linn.

Seen flying over the flowers of weeds.

*Bombus* of three species, not identified.

*Xylocopa Virginica*, Fab.

Two or three specimens were noticed flying about the windows of the wooden houses.

*Polistes Canadensis*, Fab.

The dark brown variety is common on *Baccharis* and other bushes.

*Stizus grandis*, Linn.

Solitary specimens seen alighting on the bare ground.

*Bembex* of two species noticed on the rocky soil.

*Odynerus*, sp?

Seen on the twigs of *Baccharis*.

*Formica*. A black species, not identified.

*Myrmica*. Likewise not identified.

Several forms of the group *Ichneumonidae* were noticed, but not collected nor determined.

## ORTHOPTERA.

*Gryllus luctuosus*, Serv.

This lively black cricket is quite common among the large fragments of rock, in the cracks between which it hides and finds shelter. All the specimens seen, of both sexes, had the wings very long.

The wing-covers were brown, but not so pale as in the specimens found on the sea-coast. Heat, moisture, a mild climate during most of the year, abundance of food and protection enable this insect to perfect all its organs to the utmost extent.

*Oedipoda eucerata*, Harris.

The red-winged variety may be found in bare spots where the rocks have been reduced to sand.

*Oedipoda carolina*, Burm.

Quite common all over the island; as well upon the rocks as upon the soil. It is interesting to notice how well these specimens match the dark granite amidst which they live. By close attention one may readily predict the color of the soil from which this species has been taken. On the red-sandstone soils it is of that color, on the black loams it is very dark, and upon the yellow clay soils it is of the latter color.

It does not, however, become sand-colored when found on the sandy beaches; but we have no evidence that it ever lays eggs or hatches in such places.

*Caloptenus femur-rubrum*, Burm.

Moderately common among the weeds.

*Caloptenus differentialis*, Thomas.

One specimen seen and captured.

#### NEUROPTERA.

*Epitheca filosa*, Hagen.

Several specimens were flying and gracefully balancing themselves in the air along the case-mates. The females had wings tinged with a large clear fuscous cloud towards their tips.

*Tramea carolina*, Hagen.

Two or three examples seen early in the morning flying over the weeds.

*Celithemis eponina*, Hagen.

Not common. Seen on the tops of bushes and tall weeds.

*Libellula auripennis*, Burm.

Very common. The larvae inhabit brackish pools and ditches in the salt marshes.

*Libellula quadrupla*, Say.

A few specimens were caught while they were flying over the rocks and in some cases settling upon them.

*Libellula pulchella*, Drury.

This species seems to prefer the prominent rocks and casemates as points upon which to settle and rest.

*Libellula semifasciata*, Burm.

Several specimens were captured on the tops of the tall weeds.

*Dythemis longipennis*, Hagen.

Very common upon bushes and plants. It proved to be quite variable both in size and color. The males were much more numerous than the females, and they were usually much coated with the blue powder present upon the abdomen when fully matured.

*Diplax Berenice*, Drury.

Particularly abundant, very tame, and easily taken from projecting limbs of bushes. Four well marked varieties were observed, and both sexes were variable.

*Anax junius*, Drury.

Taken on the wing. It flies wildly over the open avenues near the casemates, and but rarely alights.

*Aeschna Heros*, Fab.

Seen flying in the same places as the last, but less frequently.

*Aeschna clepsydra*, Say.

Captured on the roof of a casemate. Only one specimen seen.

*Agrion putridum*, Hagen.

Two or three specimens seen on the rocks. All of these Dragon flies came from the adjacent mainland two or more miles distant; and as we are acquainted with a large number of others from this vicinity, it seems likely that most, if not all of them, live during a part of their nomadic life upon this artificial island.

## The Development of Lingula and the Systematic Position of the Brachiopoda.

BY W. K. BROOKS.

It has been known for nearly twenty years that some of the hingeless Brachiopods pass through a free-swimming larval stage. Fritz Müller has figured and briefly described this stage of development of an unknown Brachiopod from the coast of Brazil, and Mr. Crady has given a very brief description, from memory, of the swimming larva of Lingula. These papers, which will be more fully referred to at the close of the present article, are of interest, since they establish the fact that these two forms do pass through a locomotive stage; but the papers are very incomplete, as neither of the observers traced in detail the transformation into the adult. They agree in describing the larva as furnished with a locomotive apparatus which consists of a crown of ciliated tentacles capable of more or less retraction and expansion; and in describing the animal as enclosed in a flattened orbicular bivalve shell, without a peduncle.

A more exact knowledge of the structure of the larva and of its transformation into the adult is greatly to be desired, and I was therefore much pleased to find the larva of *Lingula* in the vicinity of Fort Wool, during the past summer, in considerable abundance. I succeeded in tracing its development from a very early stage to the time when most of the adult characteristics have appeared, and my observations not only show that Mr. Crady's fragmentary account is correct in every particular, but also give us a very thorough acquaintance with the embryo. It will also be seen that there is a close general similarity between Müller's larva and the young *Lingula*, and at the same time considerable difference in unimportant characteristics.

#### GENERAL DESCRIPTION OF THE LARVA.

The free-swimming embryos of *Lingula pyramidata* (Stimpson) were met with in abundance at Fort Wool, from about the middle of July to the middle of August, and as the youngest stages were met with in the early part of this period, while only the older larvae were found at the end, it is probable that the breeding season is short. No adults were found until the end of July, and the reproductive organs did not then



present any indications of functional activity, and although a number of individuals were kept in an aquarium for several weeks no eggs were laid, and I was unable to obtain the early stages of development.

The larva, Plate 1, Figures 1 and 2, and Plate 2, Figure 3, is enclosed between two orbicular flattened valves, which are not articulated to each other, but are free around the entire circumference. The dark-colored, somewhat opaque, flask-shaped digestive organs occupy the centre of the cavity of the shell, and are in contact above and below with the integument which lines the valves. Around the digestive organs is a body-cavity bounded externally by the integument, which is continuous with the mouth above and below, and is bent downward at right angles to the valves to form the body walls. On the sides of, and behind, and in front of the body, there is a capacious mantle chamber which is open around the entire circumference. The mouth opens in the centre of a broad, flat, nearly circular disc or lophophore, around the margin of which are the ciliated tentacles. The plane of the lophophore is not at right angles to the long axis of the body, but inclined so as to be nearly parallel to it. The tips of the tentacles may be extended beyond the edges of the valves and thus form a swimming apparatus, somewhat like the velum of a mollusk,

by the aid of which the larva floats in the water or rises slowly to the surface. I have never seen the lophophore stretched out as in Müller's figure, however.

#### THE SHELL, AND THE CHANGES IN THE FORM OF THE LARVA.

The youngest stage which was studied is shown in Figure 1; it was captured with a hand net at the surface of the water, on the evening of July 22d. The figure is drawn to scale as seen with a magnifying power of 250 diameters. (Zeiss, Obj. D, Oc. 2.) The changes undergone by the larva during development are gradual, and do not involve any marked metamorphosis, and we may therefore refer the topography of the larva to that of the adult. In the figure, that valve of the shell which is uppermost, is to become the movable valve of the adult; that which Conchologists have agreed to call the dorsal valve, while the peduncle is to be attached to the opposite valve which is accordingly ventral. The nearly straight margin of the shell is that from which the peduncle is subsequently to project, and therefore indicates the posterior end of the body, while the opposite rounded margin is anterior. The right and left valves of the animal, are those which lie to the right and left respectively in this figure. The

valves are thin, brittle and transparent, and are very nearly alike in shape and size. At this stage they are somewhat wider than long, and the outline is pretty uniformly curved, except at the posterior end, which is nearly straight, and at this stage about as long as two-thirds of the antero-posterior axis of the shell. At the ends of this line are two tooth-like processes which project outwards and backward. The straight portion of the ventral valve is a little longer than that of the dorsal valve, the teeth of which *a, a*, are inside the teeth *b, b*, of the ventral valve. The surfaces of the valves are nearly flat, and the cavity between them very shallow. The thickness is so slight as compared with the width, that a floating larva, seen with a hand lens, seems to vanish when it rotates so as to present an edge view. The teeth of the dorsal valve are connected by a delicate line *c*, like the arc of a circle, of which the margin of the valve between the teeth is the chord, and with a versed sine equal at this stage to about one-third of the antero-posterior diameter of the embryo. As the straight posterior margin, the teeth and the semicircular line do not change their absolute size during the growth of the larva, and as the straight margin and teeth may be seen in the young *Lingula* after it has acquired most of the adult characteristics, these structures furnish us with a very convenient

standard, by reference to which changes in the size and shape of other parts may be readily described and properly estimated.

As the shell grows the additions take place at the sides and anterior edge, and the longitudinal axis therefore increases faster than the transverse, and the outline soon becomes circular, and then gradually elongates. At the stage shown in Figure 2, which is a ventral view, the shell is nearly circular, but a little longer than wide, while in the stage shown in Figure 3, (a dorsal view,) the longitudinal axis is considerably longer than the transverse. Since the line *aa* is of the same absolute size, at all stages of development, it will be seen that the embryo of Figure 3 is much larger and less highly magnified than that shown in Figure 2.

The body has also increased in size, and the semicircular line *c*, which in Figure 1, *c*, reached to the middle of the body, now reaches only to its posterior extremity.

As the larvae could not be made to thrive in confinement, the actual change from the free to the sedentary form was not observed, but it probably takes place soon after the stage shown in Figure 3.

The youngest sedentary *Lingula* which was found is shown in Figure 7, Plate 4, as seen from the ventral surface; and in Figure 8, Plate

5, as seen from the dorsal surface. The small vertical line at the right of Figure 7, gives the actual length of the now greatly elongated shell, which is more than twice as long as wide and pretty regularly elliptical in outline. The two valves are still about equal in size, as is shown in Figure 8, where the dorsal valve is rotated a little upon its centre, so that the axes of the two valves cross each other like the halves of a pair of scissors. At the posterior end of the shell, in Figures 7 and 8, there is an elliptical line *nu*, the edge of the *nucleus*; as this is only a little larger than the shell of Figure 3, and of about the same size, it probably shows the shape of the shell at the time when the larva settles at the bottom. It is about one-seventh longer than wide, and its anterior margin is the arc of a circle. The adult shell does not differ very much from that of Figures 7 and 8. (See the excellent figures by Morse. The Systematic Position of the Brachio-poda, by Prof. E. S. Morse, Proc. Boston Soc. Nat. Hist., vol. xv, 1873. Plate 1, Figures 2 and 3.) It is about twice as long as wide, and the anterior end is rectangular. I was not able to learn anything of the significance of the semi-circular plate shown in Figures 1 and 3. It is found only in the dorsal valve, and is either a mark upon its inner surface or a plate between the body and the valve. According to Fritz

Müller, the Brachiopod larva studied by him possessed a similar structure, which in this case was a distinct movable plate capable of being rotated backward upon the posterior margin of the shell. He says, (Reichert u. Du Bois-Reymond's Archiv., 1860, p. 74.) "Mit ihrem Hinterrande dem ausgebuchteten Hinterrande der Bauschale anliegend, gewahrt man zwischen den Schalen eine querovale Platte, 6.06 mm. lang, 0.11 mm. breit, mit dunklerem oft braunrothlich gefärbtem, ringförmigem Rande. Sie haftet an der Bauschale, deren Bewegungen sie folgt, und steht mit der Rückenschale nur durch Muskeln in Verbindung." In his second paper, (Arch. f. Naturgeschichte, 1861, p. 54.) he says that after the animal settles to the bottom. "die bis dahin zwischen den Schalen verborgene querovale Platte (der Stiel) tritt hervor, indem sie sich, wie es scheint, um dem ausgebuchteten Hinterrande der Bauschale vollständig herumdreht und so ihr vorderer Rand zum hinteren wird." In an older embryo, p. 55, he found no traces of the plate. The embryo of *Lingula* is so small and thin that if this were a separate plate, it would be rather difficult to prove without seeing it move, or finding it bent outward. In the absence of such evidence, we seem warranted in concluding that it is a similar structure to the movable plate of Müller's larva, although, in *Lingula* at least, it is

in connection with the dorsal, not the ventral valve. If it is the same, Müller is certainly in error in his suggestion that it is the peduncle, for as I shall show, there is no connection between the two structures. It will be seen that if it is a plate bent inwards from the margin of the dorsal valve, it agrees closely in position and connections with the complicated structure which in the articulated Brachiopods, forms the hinge plate, crura, and loop which supports the arms.

Before I pass to the description of the internal organization of the larva, I wish to call attention briefly to the fact that the recent and fossil shells of the various species of *Crania*, *Discina*, *Lingula*, *Lingubella*, *Obolus*, and other hingeless Brachiopods, furnish a series of adult forms representing all the changes through which the outline of the shell of *Lingula pyramidata* passes during its development.

#### THE DIGESTIVE ORGANS.

In a dorsal view, at the earliest stage which was studied, Figure 1, the digestive organs are quite conspicuous since they form a somewhat opaque mass which fills the posterior half of the ventral portion of the otherwise quite transparent embryo. In a dorsal view at this stage, the digestive tract is very similar in shape to a long-necked round-

bottomed Florence flask; the œsophagus corresponding to the neck, while the stomach represents body of the flask.

The thick walled muscular œsophagus, Figures 1 and 4, *i*, is lined with cilia and runs forward from the stomach on the median line, nearly to its anterior end, where it bends sharply at right angles, towards the ventral surface. The mouth accordingly has a ventral aspect, and in a dorsal view, Figures 1, 3, 4 and 6, *o*, it is seen through the bend of the œsophagus, while in a ventral view, Figures 2 and 5, *o*, its surface is shown. It is widely open, and is ciliated like the œsophagus, and small food particles are continually drawn into it and whirled down the open œsophagus into the stomach, *k*, *l*. The latter is a large, permanently dilated ciliated thick-walled pouch, and is imperfectly divided into two chambers. The one nearest the mouth, Figures 1 and 2, *k*, is the largest, and its walls are filled with dark yellow, highly refractive oil-like globules. This cavity I shall speak of hereafter as the oral chamber. It usually contains a mass of partially digested food, which occupies the centre of the cavity; does not touch the walls, and is kept in constant slow rotary motion by the ciliary lining. Near the bottom of this chamber the walls are a little raised so as to form an elevated line; the lower border of what is to become the liver of the adult, which is divided at this stage into two



lobes, *n*, Figure 1, which are separated from each other upon the median line, of about half the dorsal surface of the oral chamber of the stomach. The posterior end of the stomach is formed by a second chamber, which is nearly spherical, with thick transparent ciliated walls, without oil globules, *l*, Figure 1. This chamber I shall call the intestinal, since it communicates with the intestine. It is only imperfectly separated from the oral chamber, but owing to the opacity of the walls of the latter, I was not able to make out the exact form of the opening between the two.

The intestine, *m*, Figure 2, (ventral view,) originates upon the ventral side of the posterior end of the intestinal chamber, and passes forwards, ventral to the stomach, to the anus, *n*, Figure 2, which is upon the right side of the body.

The intestine was present in the youngest larva which was carefully studied, and in Figure 1, its edge is shown at *m*, projecting beyond the margin of the stomach, but at a somewhat later stage, Figure 2, its cavity was not yet in communication with the cavity of the stomach, and there was no external opening. As Müller speaks of a stage in which it was wanting in his larva, it probably originates as a solid outgrowth from the posterior end of the stomach; meets and unites with the integument; becomes hollow; establishes a communication with the stomach, and finally opens externally by an anus.

In the ventral view, Figure 2, the distinction between the oral and intestinal chambers of the stomach is much less pronounced than upon the dorsal surface, and the oil globules come to an end irregularly below, without any sharp boundary. The digestive organs undergo very little change during development, and in the young sedentary *Lingula*, Figures 7 and 8, they have substantially the same form as in Figures 1 and 2. In Figure 4, they are shown in dorsal view, at about the same stage as Figure 2, but more highly magnified. In Figure 3, a somewhat older larva is shown in a dorsal view, and Figure 5 is a more magnified ventral, and Figure 6, a dorsal view of the body and viscera of the same larva. In the latter figures, it will be seen that the liver *h, h*, is now sharply folded off from the stomach below, and definitely limited along the lower margin, in both the ventral and dorsal aspects. In Figure 5, it will be seen that the intestinal chamber of the stomach, *l*, is now more definitely separated from the oral chamber *k*, which is now entirely covered by the liver.

The intestine *m*, is now quite conspicuous, and its cavity is filled with fæces, some of which were observed to pass out through the anal orifice, *n*.

By a comparison of Figures 1 and 2, it will be seen that the œsophagus opens into the stomach near its dorsal surface, and the dorsal surface of

the œsophagus is accordingly quite near the dorsal valve of the shell, while its ventral surface is quite removed from the ventral valve, and separated from it by a space which is part of the mantle cavity, and contains the ventral edge of the lophophore. This arrangement of parts is shown in the diagrammatic longitudinal section, Figure 16, Plate 6, where the dorsal valve is upon the right side.

#### THE BODY-CAVITY, OR PERIVISCERAL-CAVITY.

Surrounding the digestive organs is a body cavity, *g*, Figures 1, 2, 3, 4, 5, 6, 7 and 8, which is limited externally by a muscular integument, (*f*) or body-wall. This cavity is quite narrow in the younger larvae, and becomes more capacious as development progresses, but its size is very variable, owing to the contractility of the body-wall. In Figure 4, it is almost obliterated, and the integument is in contact with the surface of the digestive tract except around the bottom of the œsophagus, where the cavity *g*, is still visible. In another figure (2) of the same stage the body cavity is quite conspicuous, and in a younger embryo, (1) it was recognizable around the whole stomach. This difference is due in part, to the fact that Figure 4 was drawn from a larva which was somewhat compressed, but the body-wall was

often observed to expand and contract to an almost equal degree.

As shown in Figures 5 and 6, the inner surface of the integument is richly ciliated, and the body-cavity is filled with a corpusculated fluid which is kept in constant motion by the action of the cilia, and by the contractions of the integument. No cilia were visible upon the outer surface of the digestive tract, and they are probably restricted to the integument.

In the adult, this body-cavity is pretty completely filled by the muscles and reproductive organs, and its relations are consequently obscure, but in the larva shown in Figure 8, which has acquired most of the adult characteristics, its arrangement is substantially the same as in the larva, and the integument is contractile.

#### THE BLOOD.

Whenever a considerable quantity of the corpusculated fluid is massed together, it is seen to have a distinct, but faint, violet color, and in such accumulations many of the corpuscles were observed to have a singular dancing motion which did not seem to be due to the contraction of the body-wall, or to the action of its cilia.

The indications of an independent power of motion in the corpuscles themselves, were in some cases so marked, that I attributed them for some time to the presence of parasitic infusoria in the body-cavity, and I even thought that what I had regarded as blood corpuscles might be parasitic organisms. The appearance could be seen at all stages of growth whenever the corpuscles accumulated, and upon examining some of the fluid from an adult *Lingula*, I found it to contain two kinds of corpuscles, in about equal numbers, as shown in Figure 12, Plate 6. One kind are small, colorless, nearly spherical, and usually quite granular; their surfaces are frequently roughened or covered with projecting knobs. They exhibit slight changes of form, the knobs making their appearance upon the smooth surface of a corpuscle, and again gradually disappearing. Many of the smooth corpuscles of this kind have a cup-shaped depression upon one side.

The other sort of corpuscles are very peculiar; they are much larger, vary greatly in size and shape, but are usually more or less fusiform. In a thin stratum they appear colorless, but aggregations have a faint violet tinge. They consist of transparent protoplasm without granules, and with no visible nucleus. Running out from one or both of the pointed ends of many of the corpuscles are long delicate filaments of variable length;

frequently four or five times as long as the enlarged portion. These flagella were wanting in many of the corpuscles, and in one or two cases they were seen to gradually disappear, as if they had been retracted into the substance of the corpuscle. The blood, when removed from the body, was perfectly motionless; the dancing motion having stopped, and none of the flagella were seen in motion, and it is possible that the movements of the corpuscles in the body-cavity are entirely due to the action of the cilia of the integument. It certainly did not have that appearance, and, as I have said above, my attention was attracted by the movements before I found the flagella. I think, then, that there is no reason to doubt that the flagella are motile, and give to the corpuscles a proper motion.

Fusiform corpuscles in process of division into two were numerous, and several were shown in the figure. Morse (Systematic position of the Brachiopoda, p. 25,) has called attention to the presence of these two kinds of corpuscles in the perivisceral fluid of *Lingula*, but he attributes the color of the fluid to the round corpuscles.

## THE MANTLE AND INTEGUMENT.

During the earlier stages of development the mantle is quite transparent, except around its circumference, where it forms a thickened granular opaque rim, concentric with and near the margin of the shell. (See Figures 1 and 2.) The rim is at first of uniform width, but at the stage shown in Figure 3, it is much wider at the anterior end and sides than posteriorly, and setae are now developed in it, pointing towards and protruding from the margin of the shell. These are at first sparsely scattered, but soon become more numerous, and at the stage shown in Figure 8, they are not of equal length; those near the posterior end of the shell being quite long; those in front of them much shorter; those about half way between the middle and the anterior end long, as well as those at the anterior end. They are movable and readily drop off, and in the adult they appear to be continually dropped and renewed, since the bottom of the vessel in which adults are kept alive, soon becomes covered with them.

At the stage shown in Figures 7 and 8, the anterior portion of the mantle is quite thick, and forms a broad opaque pad, upon which, in each valve, are four dark brown pigment spots; two smaller ones near the median line,

Starting at the point *d* on the dorsal surface in Figure 1, we have the integument running back only to a point a little beyond the letter *e*, and then bending down and forwards onto the lophophore, as shown on the right side of the diagram, Figure 16; so that the dorsal aspect of the mantle chamber is quite shallow. The portions of the mantle chamber at the sides of the body communicate posteriorly behind the body, and floating particles were seen to pass from one to the other without passing outside the shell; but as the mantle is here greatly thickened, the mantle chamber appears in surface view to be divided by a partition.

#### THE PALLIAL SINUSES.

During the earlier stages the two layers of the mantle are closely united outside the limits of the body, forming a solid mantle fold, without vessels or other visible cavities; but at about the stage shown in Figure 3, four diverticula from the body-cavity make their appearance, and penetrate between the two layers of the mantle, one on each side of the body in the mantle of both the dorsal and the ventral valve. They rapidly increase in size and form two long horn-like chambers on each surface of the body, Figures 5 and 6, *v*, which run forwards within the mantle,



nearly to the anterior margin of the valve. They communicate with the body-cavity by large ciliated funnel-shaped apertures, Figures 5 and 6, *w*, and they are richly ciliated internally, and the blood corpuscles are constantly driven into and drawn out of them by the irregular contractions of the body-wall. There is thus a very perfect circulation of the blood in these chambers; the muscular integument performing the function of a pulsating heart.

Soon a second diverticulum makes its appearance, running back from the posterior margin of the first, near the point where the latter bends down to open into the body-cavity. This second chamber, which is shown, but not lettered in Figures 7 and 8, extends back towards the posterior margin of the shell, and like the first is ciliated and filled with blood. The outer margins of these "pallial sinuses" now give rise to numerous small diverticula which lengthen and project downwards into the pallial cavity, as little papillae, or "ampullae," the well known respiratory organs of the adult *Lingula*. The fact that some of the earlier students of the anatomy of the Brachiopods figured and described from alcoholic specimens a heart and a system of blood vessels, has given rise to much confusion in regard to the blood circulation in the group; but the labors of Semper, (*Z. Z.*, xiv, p. 424); MacDonald, (*Trans.*

Linn. Soc., xxiii. p. 373). and Morse, (Systematic position of the Brachiopoda, p. 24). and others have satisfactorily settled the question, and shown that there are no distinct blood vessels and no heart, and that the circulation is due in part to ciliary action. My observations are, so far, only corroborative of this now generally accepted view, but as these observers studied only the comparatively large and opaque adults, they overlooked the important part played by the integument in propelling the blood.

#### THE MUSCLES.

Almost every naturalist who has written upon the anatomy of the Brachiopoda, has proposed a new name for each muscle, and the confusion thence arising is much to be regretted. In order to escape the employment of a cumbersome synonymy, it seems most convenient to make use of arbitrary letters to designate the muscles instead of descriptive names, and, in order to facilitate comparison with the myology of the adult, I shall call the muscles of the adult *Lingula*, by the letters which mark them in Figures 23, 24 and 25, of the article "Brachiopoda," by Davidson, in the *Encyclopædia Britannica*, Ninth Edition.

In the youngest stage observed, only a single pair of muscles *p*, Figure 1, were present. They

are situated on each side of the œsophagus, close to its union with the stomach, and traverse the body cavity from one valve to the other. They are the muscles "*h*," of the article "Brachiopoda," and by their contraction bring the two valves together, compressing the body between them. The separation of the valves appears to be accomplished at this stage entirely by the muscular integument. When the muscles "*h*," are relaxed and the lateral walls of the body contracted, the width of the body is diminished and its thickness increased, and the valves thus pushed apart. These muscles were found at all stages, and are shown at *p*, in Figures 1, 2, 3, 4, 5, 6, 7 and 8. At a somewhat later stage, Figure 2, the integument at the posterior end of the body is much thickened, and soon a band of muscular fibres, *r*, make their appearance in it, upon the median line, and run directly from one valve to the other. At the stages shown in Figures 4 and 3, the fibres have increased in numbers, and form a well marked muscular bundle, *r*, which is shown in all the succeeding figures, and is the muscle "*g*."

When contracted at the same time with the muscles "*h*," this muscle serves to flatten the body still more, and this brings the two valves closer together; but when the muscles "*h*" are relaxed and the muscle "*g*" alone contracted, the posterior margins of the valves are brought

together and their anterior edges thrown apart, the body furnishing the fulcrum around which they turn.

At about the stage of development shown in Figure 3, three pairs of muscles make their appearance in the body-cavity at the sides of the stomach, two on the dorsal and one on the ventral side. They are shown at *u*, *u'* and *u''* in Figures 5 and 6. In Figure 5, the ventral ends of one pair are seen at *u* attached to the shell inside the limits of the body-cavity on each side of the posterior end of the hepatic chamber of the stomach. Dorsally they run forwards, and appear to unite with the dorsal ends of the muscles "*h*," as no independent ends of similar muscles were visible in a dorsal view. They are the muscles "*j*" of the adult.

In a dorsal view of the same larva, Figure 6, the dorsal ends of two pairs of muscles are seen at *u'* and *u''*. They are attached to the shell a little outside the limits of the body, and the integument bends out with them at *z*. One pair of them, *u'*, run forwards and downwards, and apparently unite with the ventral ends of the muscles "*h*," for no corresponding independent muscles could be seen in a ventral view. In Figure 7, the ventral end *p* of the muscle "*h*" is plainly seen to be divided into two by a longitudinal line, and a comparison with the Ency-

clopædia figures will show that the outer half is the ventral end of the muscle *u'* of Figure 6. This muscle apparently corresponds to the two muscles "*k*" and "*l*" of the adult, and it probably divides into two at a later stage. It would appear, then, from these observations, that the muscles "*j*," "*k*" and "*l*" of the adult *Lingula* are derived from the muscle "*h*," some of its fibres separating from it at the ventral end to form the muscle "*j*," but remaining for some time united to it dorsally; while another bundle separates from the dorsal end, but remains attached for some time ventrally, and gives rise to the muscles "*k*" and "*l*."

According to King and Davidson, these three pairs of muscles are adapted to move the valves forwards and backwards upon each other, but as my observations show that neither the larva nor the adult does slide its valves backwards and forwards to any marked degree, it seems more probable that they, together with the pair of muscles next to be described, cause the rotation of one valve upon the other, around the body as a centre.

In the dorsal view, Figure 6, a part of the muscles *u'* are shown, attached to the shell close to the muscles last described, and running backwards and inwards under the stomach. They are the muscles "*i*," which, in the adult, cross each

other under the digestive organs, and are the principal causes of the rotation of one valve upon another, which has been described by Semper and Morse, and which is represented in Figure 8.

We thus have, at the stage shown in Figure 3, and while the larva is yet a free swimming animal, something to represent all the muscles which have been described in the adult; and, in addition to them, I find upon the ventral surface a pair of muscles, Figure 5, *yy*, which have never been described. They run from the anterior margin of the stomach to the posterior end of the body, where they unite with the mantle, close to the ventral end of the muscle "*g*." They appear to be adapted to pull the oesophagus and lophophore back into the shell, and may therefore be spoken of as *retractor muscles*.

In the more advanced stages shown in Figure 7 and 8, the body-wall was too opaque to allow the muscles to be seen in a surface view, and the sections which I have made of the young *Lingula* at this stage will be described in another place. I may say, however, that these sections show that the view given above as to the adult homology of the muscles of the swimming larva is correct.

The muscles of the peduncle will be spoken of in connection with that organ.

Running along the dorsal surface of the stomach in Figure 6, is a thickened ridge 1, which appears to connect the stomach with the lining of the dorsal valve. It appears to be solid, and it certainly exhibited no pulsations or contractions, so long as the shell of the larva remained sufficiently transparent to allow it to be examined.

#### THE NERVOUS SYSTEM AND SENSE ORGANS.

During the later stages, Figures 7 and 8, the shell and body-wall were too opaque to permit of the minute study of underlying structures, and at the stage shown in Figures 1 and 2, the nervous system was very imperfectly developed, and I will therefore speak first of the stage in which it was most satisfactorily studied, that shown in Figures 3, 5 and 6. At this stage it consists of a commissural ring  $x'$ , Figure 6, which encircles the œsophagus, at its union with the stomach, and carries one ventral ganglionic enlargement, Figure 5,  $x$ , and two lateral ganglia, Figure 6,  $x''$ , and two dorsal otocysts, Figure 6,  $x'''$ .

The ventral median ganglionic mass, shown at  $x$ , in Figures 2, 3, 5 and 7, is the largest and most conspicuous portion of the nervous system, as well as the first to make its appearance in the larva. It is situated upon the inner or posterior face of

the fold of integument which forms the anterior wall of the body, and which runs from the ventral valve to the base of the œsophagus, as shown in the diagram, Figure 16, *q*. It is formed by a thickening of the integument, and there is no line separating the nervous from the integumentary portion, and while the rest of the nervous system is separated from the body-wall, and lies upon the surface of the digestive tract, this, the first portion to make its appearance, is permanently united to the integument, and as we shall see that the rest of the nervous system grows out from it on each side, it is plain that after the analogy of most animals in which embryonic history has not been greatly modified by adaptation, the nervous system of *Lingula* is ectodermal in its origin.

At the earliest stage observed, this ganglion was present, and a little later that the stage shown in Figure 1, its ends are prolonged into two fibres, which are free from the integument, and bend around the œsophagus. At first they reach only about half way round the œsophagus, and end in the two lateral ganglia *x'*, Figures 4 and 6.

These are transparent, slightly granular, and without pigment, although Müller figures and describes in his larva, (Reichert u. Du Bois-Reymond's Archiv., 1860, Taf. 1, B. Fig. 1,) a pair of brown pigmented eye spots, which are identical in position with these ganglia of *Lingula*,



and are no doubt the same structures. At about the stage shown in Figure 2, the nerve fibres have lengthened as shown in the dorsal view, Figure 4, and now reach to the dorsal surface of the stomach upon which they lie. They now terminate in a pair of large dilatations, Figures 4 and 6, *x'''*, each of which contains an elliptical cavity, within which are numerous vibrating otoliths. These organs were figured and described by Müller in 1860, and to him, therefore, belongs the credit of first discovering them. I am unable to agree with Morse in his view that the otocysts exhibit any annelidan characteristics; they are precisely such structures as are found in most of the Mollusca, and in numerous other animals of the most diverse forms, and are absolutely without morphological significance, in the present state of our knowledge, at least. At the stage of development shown in Figure 3, the otocysts are connected by a dorsal commissure, and the nerve-ring is thus a complete circle surrounding the oesophagus. In the young sedentary *Lingula* the shell is too opaque to allow the nervous system to be examined carefully, but in a ventral view, Figure 7, *x*, the ventral ganglion was visible, and in a dorsal view, Figure 8, *x'*, the dorsal portion of the ring. It should be recollected that Figure 8 is much larger and less highly magnified than Figure 3, and if the otocysts had been present,

and no larger than those of the latter figure, they would not have been visible with any available magnifying power. There is no reason, therefore, to suppose that they disappear during development.

#### THE PEDUNCLE.

In the youngest larva which was studied, Figure 1, there is no trace of the peduncle, and the posterior end of the body is attached by a membrane formed by the thickening of the integument to the posterior margin of the mantle. At about the same time that the muscle *r*, Figure 2, makes its appearance, the peduncle *s* also appears, a little to the right of the posterior end of the body, and it is soon seen to be a hollow structure, surrounded by a double wall. When more highly magnified, Figure 9, Plate 6, the two layers which enter into its wall are quite distinct, 1 and 2, and the large central space is seen to be ciliated, and contains blood-corpuscles. These are driven into and out of the cavity of the peduncle by the motions of the body, and there is evidently a free communication between the body-cavity and the cavity of the peduncle. When seen from the dorsal surface, Figure 9, the outline of the peduncle is complete, and it is necessary to examine the ventral surface in order to study its connec-

tion with the body. Figure 10, Plate 6, is a ventral view of the posterior portion of the body and digestive organs of a larva of about the same age as that shown in Figure 2, and Figure 9 is a dorsal view of another larva of about the same age. The ciliated integument *f*, the body-cavity *g*, the intestinal chamber of the stomach *l*, the intestine *m*, and the muscle *r*, are lettered as in the preceding figures.

In Figure 10, it is seen that the inner layer, 2, of the peduncle no longer forms a complete tube, but flattens out as it approaches the posterior end of the body, and appears to become continuous with the muscle *r*, while the outer layer, 1, becomes continuous with the integument of the posterior end of the body. It will be observed that the ciliated epithelium which lines the body-cavity, is anterior to the muscle *r*, and that this muscle lies between the two layers of epithelium which form the outer and inner faces of the integument.

These facts seem to indicate that the peduncle is simply a diverticulum from the posterior end of the body, formed by a tubular outgrowth from the integument, with a cavity which is in communication with that of the body.

The ciliated epithelium of the inner surface of the peduncle is continuous with that of the body, its outer layer is continuous with the integument, and between this and the ciliated epithelium is

a circular layer of muscles continuous with the muscle *r*.

The peduncle now rapidly increases in length and soon bends abruptly upon itself, and passes behind the body so that its free end, *s*', now lies upon the left side, as shown in Figures 11 and 3. Its free end now becomes somewhat enlarged, and as it continues to increase in length, it becomes sharply bent upon itself at short intervals, as shown in Figure 3. As long as the larva swims freely in the water it is coiled up within the shell, and although it is somewhat contractile, and changes its shape, it is never extended. The contraction of the circular muscles of its wall at somewhat regular intervals, give it a wrinkled appearance, which has been mistaken by Morse, (Systematic position of the Brachiopoda, p. 11.) for segmentation.

The wrinkles are, however, not permanent, and I have been unable to find, at any stage of development, any indication that the peduncle is made up of segments.

We cannot fail to mark the contrast between the appearance of the peduncle at a very early stage of development in the hinged Brachiopods, according to the observations of Lacaze-Duthiers, Morse and Kowalevsky, and its appearance at a very much later stage in the hingeless forms as observed by Müller, McCrady, and myself.

If, as I shall try to show, the form of development which we have in *Lingula*, is the unabridged record of the ontogeny of the group, and that of the hinged forms an accelerated form of development, it is plain that the Brachiopodan peduncle is a structure which was acquired after that stage of ontogenetic development which is represented by the free unpedunculated larva, and is accordingly of no morphological value, so far as the question of the systematic position of the Brachiopoda is concerned.

#### THE LOPHOPHORE AND TENTACLES.

During the earlier stages the lophophore is nearly flat, Figures 1 and 2, *q*, and is inclined to the long axis of the body in such a way that its dorsal margin is anterior, and its ventral margin posterior to the mouth, which is, itself, ventral to the long axis of the oesophagus. In the diagrammatic longitudinal section, Figure 16, Plate 6, the thickness of the larva has been, for convenience, greatly exaggerated, as compared with the length, but a comparison of Figures 1 and 2, will show that the position of the lophophore is as it is represented in the diagram.

The tentacles which are arranged around the margin of the lophophore, increase in number

with the age of the larva. In Figure 1, there are only five pairs, and in Figure 7, sixteen pairs.

Only one of the tentacles is upon the median line; that which is dorsal to the mouth, and which is indicated by the letter *d*. The new tentacles appear in pairs at the base of this odd one, and are shown at *d'* in Figures 1, 2 and 3. As new tentacles are added, the old ones are pushed away from the median dorsal one, and crowded down towards the ventral margin of the lophophore, and in Figures 1, 2, 3 and 7, the series of letters *d, d', d'', d''', d''''*, etc., indicate the relative ages of the tentacles, the single dorsal one being the oldest, and the one nearest the ventral surface, the oldest pair. As shown in Figure 2, *d'*, these are widely divergent from each other, and the lophophore is thus relatively symmetrical or hippocrepiian from the inside as in the hippocrepiian Polyzoa, the median convex surface of the horse-shoe is upon the side of the body where the anus and nephridia are situated. At an early stage, Figure 1, the antero-posterior diameter of the lophophore is about equal to the transverse, but as the number of tentacles increases, the sides of the lophophore lengthen as in Figure 2, where there are eight tentacles on each side. As the number of tentacles increases, those at the ends of the lophophore are pushed out of the

series, and bend towards the dorsal surface, and in Figure 3, (dorsal view,) where there are nine pairs, this dorsal flexure is seen in all from the fifth,  $d^5$ , to the eighth,  $d^8$ , and the outline of the lophophore is no longer the same in a dorsal and a ventral view. In Figure 7, where there are sixteen pairs of tentacles, and in Figure 8, where there are eighteen, not only the tentacles, but the lateral edges of the lophophore are bent towards the dorsal surface, as shown at  $q$ , Figure 8. As the number of tentacles continues to increase, the lateral edges  $qq$  continue to lengthen, and thus form the lateral coiled "arms" of the adult, while the dorsal margin also lengthens, bends towards the dorsal surface and forms the median coiled "arm." The tentacles become the cirrhi of the adult, and are substantially the same in structure in the larva and in the adult. In Figure 14, the tip of a tentacle, and in Figure 15, the bases of three from the young *Lingula* shown in Figure 8, are figured, and in Figure 13, a number of tentacles, much less magnified, from an adult. Like the tentacles of a polyzoan, they are covered with cilia, and can be moved in all directions, and they also resemble the polyzoan tentacles in having no power of contraction and extension like those of a Hydroid. In the larva, when they are at rest, their positions are constant, and are carefully drawn in Figures 1, 2 and 3,

which will give a better idea of their peculiar arrangement than a description. They are hollow, as shown in Figures 14 and 15, and the wall is made up of two distinct layers, of which the inner alone appears to be muscular. Owing to the varying contraction of the muscles, the tentacle exhibits the same irregularly segmented appearance as that noticed in the peduncle.

The cavity of the tentacle appears to be filled with a fluid, but this is perfectly transparent, and contains no corpuscles, and does not appear to be connected with the body-cavity. Near the bases of the tentacles the outer layer and the cilia pass over to the adjacent tentacles, while the muscular layer and cavity extend for some distance into the lophophore, as shown in Figure 15.

#### THE OBSERVATIONS OF PREVIOUS WRITERS.

The larva of the hingeless Brachiopods has been studied to some extent, and accounts published by Fritz Müller and by McCrady. The embryo described and figured by Fritz Müller, (*Beschreibung einer Brachiopoden larva, von Fritz Müller, in Destero, Brasilien. Reichert u. Du Bois-Reymond's Arch., 1860, pp. 72-80, Taf. 1, B.*



Figures 1-3,) bears a very close resemblance to that of *Lingula*, although it presents many interesting points of difference. The larvae were found floating in the water, and as they could not be raised in confinement they were not traced to the adult form, and we are ignorant of the group of Brachiopoda to which they belong, but as the author also collected at the same place the shells of *Crania* or of some allied form, (see note, by Max Schultze,) it is probable that they are the young of the same species. The animal is enclosed in a flat bivalve shell, nearly similar in outline to that shown in Figure 1, of the present paper, but unlike *Lingula*, the dorsal valve is much larger and more arched than the ventral, beyond the margin of which it projects around the entire circumference. As in *Lingula*, the shell is thin, flexible, horny and transparent. At the place of the hinge a small oval plate lies transversely between the valves. The central portion of the mantle is thin and transparent, and thus, as in *Lingula*, forms a central clear space, which is surrounded by a thickened, somewhat opaque rim.

In the larval *Lingula* at about the same stage of development, this rim is not furnished with setae, but in Müller's larva there are numerous setae, of two kinds. The smaller ones are numerous and hair-like, and appear to correspond to

those which are developed in *Lingula* at a later stage. They project from the marginal rim of the dorsal valve, and bend down and hook on to the edge of the smaller ventral valve. The second kind of setae are very much larger, and are only ten in number, five on each side, and the surface is serrated in the largest pair.

Occupying, as in *Lingula*, the posterior half of the clear area is the body proper, rounded anteriorly, and attached by its entire upper and lower surfaces to the shell.

The long, muscular oesophagus leads into an opaque flask-shaped stomach, which is covered with hepatic cells, and seems to correspond to only the oral chamber of the stomach of *Lingula*. In the larva which is figured, there were no traces of an intestine, but at a later stage of development this was seen to run forwards from the posterior end of the stomach, to open by an anus on the right side of the body as in *Lingula*. The anterior end of the oesophagus has a ventral flexure, and the mouth is in the centre of the lophophore as in *Lingula*, but unlike *Lingula*, it is placed upon an elevation or mouth papilla.

The anterior half of the clear area of the shell is almost entirely occupied by the four pairs of arms, which are arranged around the edges of the lophophore which, as in *Lingula*, is placed obliquely to the long axis of the body, with its

anterior margin dorsal, and its posterior margin ventral to the mouth. The tentacles appeared to be hollow and were covered with cilia, and arranged in pairs on each side of a median dorsal tentacle, which is much shorter than the others, terminates in an enlarged brownly pigmented knob, and gradually disappears as development goes on. In *Lingula*, this tentacle is like the others, and is persistent.

Near the anterior end of the body, on each side of the stomach, are two dark brown oval eye spots; the long axis of the oval running obliquely outwards and backwards. No such eye spots were to be seen in the *Lingula* larva, but the lateral ganglia shown in Figure 6, *x'*, are in about the same position, and may be the structures which are supplied with pigment in Müller's larva.

The otocysts are figured and described substantially as they occur in *Lingula*, although Müller failed to trace the nervous system.

The larva is able to protrude the lophophore and tentacles from the shell, and in this position, as figured by Müller, it has a very striking resemblance to a *Polyzöon*. He says that it is also able to move along the bottom; sliding the valves of the shell over each other, and thus progressing by the aid of the large projecting setae.

He figures only one stage of development, and his account of the structure of the larva at this stage is fragmentary and incomplete, but it is enough to show that there is an essential similarity between it and *Lingula*.

He concludes the paper as follows, (p. 79:)

"Die Bedeutung unserer Larve für die systematische Stellung der Brachiopoden näher zu erörtern, muss ich mich enthalten, da ich die neueren Forschungen über Bryozoen nur durch Jahresberichte kenne, und ich selbst nur wenige Formen derselben ziemlich oberflächlich untersucht habe. Dem Eindruck des ersten Anblicks folgend, würde gewiss Jeder, der unser Thier lebend zwischen lebenden Muschellarven und Cellularien gesehen, ihm ohne Bedenken seine Stelle zur Seite des letzteren anweisen. Was dabei zunächst als ähnlich im Auge fällt, die kreisförmig gestalteten Tentakel, steht in auffallendem Gegensatz zu der Armbildung der erwachsenen Brachiopoden. Aber ob überhaupt unser Thier als Larve einer der bekannten Brachiopodenformen angehört, und nicht vielmehr p. noch unbekannten Repräsentanten einer neuen Gruppe mit kreisförmig gestalteten Armen, die dann in ähnlicher Weise den Meeresbryozoen mit Tentakelkranz entsprechen würde, wie die gewöhnlichen Brachiopoden den zweiarmligen Bryozoen des süßen Wassers?"

Our knowledge of the development of *Lingula*, shows that the resemblance which is suggested between the larva and a Polyzöon is fully born out by a more careful study of the organization of the larva. As regards the suggestion that Müller's larva may be the young of an unknown form of Brachiopod with the cirrhi arranged in a circle round the mouth, our observations have shown that the facts do not warrant any such conjecture. In both *Lingula* and Müller's larva, the lophophore is bilaterally symmetrical with reference to only one axis, the antero-posterior, and the

tentacles are arranged symmetrically in pairs, and there is an approach to the hippocrepian type from the first.

It is, of course, possible, although not at all probable, that it is the larva of an undescribed species; but there is not the least reason for supposing that this species is essentially different from *Lingula*.

During the two years following, Müller repeated his observations upon the larva, which was found during the late summer months. In 1861, he published a second paper, which contains an account of the habits of the larva, as well as a few new observations regarding its structure, but no new figures. (Arch. f. Naturgeschichte, 27, 1861, pp. 53-56.)

This paper was translated in full in the Ann. & Mag. Nat. Hist., 3d Series, vol. viii, p. 505, and for convenience I make the following extracts from the translation:

“When the little animals are placed in a good sized vessel of pure sea water, they slowly ascend; the slightly gaping shells stand perpendicularly, the hinge margin downward; close to the anterior margin the right arms spread out horizontally, like rays, with their lips slightly bent downwards, and the roundish knob situated between the uppermost pair projects beyond the plain of the arms. In this posture they move slowly about near the surface. When strongly shaken, or sometimes without any perceptible reason, they retract the arms and close the shells, when they slowly turn over and sink to the bottom, with the free margin downwards. If the arms be again protruded, the hinge margin also again turns downwards. The duration of this state never exceeds five or six days, and in general, the larvae adhere to the bottom or sides of the vessel in a still shorter time. When they adhere to the sides, the mouth

was always directed downwards; the ventral shell was strongly drawn forward until its anterior margin reached or passed that of the dorsal shell. . . . For a day or more, the animal remains contracted and quiet; then the shells being slightly opened, the arms are half extended, and strike inwards, one or more at a time, just as in the marine Bryozoa. The principal change in the soft parts consisted in the retrogression of the organs of sense. The eyes had become broken up into groups of about ten black points; the previously spherical auditory vesicles were shrunken into longish sacs, closely surrounding the otoliths. In somewhat older animals there was no trace of the organs of sense, although they had not lost their sensibility to light. . . ."

He notices the gradual disappearance of the larger setae, and the formation of an intestine and anus upon the right side of the body, but he does not mention any change in the shape of the lophophore, or in the number of tentacles, or in the general form and organization of the body.

In the American Journal, 1860, p. 151, there is a short description by McCrady, of an embryo, which was correctly regarded by the author as the young of *Lingula*. He says:

"I have within a few days discovered an embryo which has at once so many affinities with the Bryozoa and the Brachiopoda, that I believe it to be an embryo Brachiopod, and very probably the young of *Lingula* pyramidata. It is an equilateral thin hyaline, straight bivalve shell, elliptical in outline, and with the valves very flat. Through this perfectly transparent shell is visible a lining membrane, or rather the borders of such a membrane, which is the mantle. Within this, and near the hinge, is a large flask-shaped body containing a digestive cavity, surrounded by a dark mass. This cavity extends into the neck of the bottle-shaped cavity,—the oesophagus—and terminates towards the gap of the shell, in a mouth. From the opposite or basal end of the digestive cavity goes off a pretty long intestine, which turns first to the left, makes several convolutions, and terminates in an anus between the valves of the shell."

It will be seen from this description that McCrady mistakes the peduncle for the intestine.

"The mouth lies on a somewhat triangular prolongation of the body-wall, which rests with its apex towards the gap of the shell, in the dorsal valve. The borders, right and left of the homologue of the arms, are fringed, each with about six cirrhi, the hindermost being the longest. The animal thus constituted, when quiet, withdraws its whole body, cirrhi and all, within the bivalve shell, which is tightly closed; but when in motion the shell is distinctly opened, and the gap of the valves is plainly visible, even to the naked eye. Through this aperture are thrust out the cirrhi, about twelve in number, which then arrange themselves in a circular funnel-like manner, precisely as in a Bryozoa polyp and by a plainly allied, and with its cirrhi or tentacles thus extended, the animal swims through the water with considerable rapidity. The cirrhi of the embryo, I take to be the homologue of the cirrhi of Brachiopoda and of Cristatella, and of Bryozoa generally. The rest of the structure, especially of the anus on the right, and the shape of the shell point, I think to Lingula embryo with cirrhi extended, about a line in length. There is no trace of a peduncle. It appears to me that this must set at rest all difficulty about the approximation of the Brachiopoda and Bryozoa, as proposed by Agassiz, and others."

McCrady promised a more extended illustrated paper upon the subject, but owing to the destruction of his notes and drawings during the war, this never appeared.

In Morse's paper upon the Embryology of Terebratulina, p. 261, a second brief memorandum, drawn up by McCrady, is printed.

Although his account is too brief to give any very exact idea of the larva, it is clear that almost every statement made by McCrady, agrees with the observations detailed in the present paper, and to him belongs the credit of first describing the locomotive Polyzöon-like larva of Lingula.

THE BEARING OF THE DEVELOPMENT OF LINGULA  
UPON THE SYSTEMATIC POSITION OF THE  
BRACHIOPODA.

A comparison of the more recent writings of a few of the standard authors upon general zoölogy, will show that the greatest diversity of opinion still exists, regarding the affinities and systematic position of the Brachiopoda. As long as the attention of the investigator was confined to the study of shells, there seemed to be no difficulty in connecting the Brachiopoda with the Lamellibranchs through such forms as *Anomia*. Although the slightest anatomical knowledge is sufficient to show that the resemblance between these forms is entirely superficial, and absolutely without scientific value, and although embryological investigations give the most complete confirmation to the conclusions founded upon anatomical research, this old view, first propounded by Linæus, is still adhered to by some conchologists. (See, for instance, Woodward's *Manual of the Mollusca*, ed. 1868, p. 5.)

Tracing the history of the subject, we find this view replaced by another which is not open to the charge of superficiality, since it is based upon



a thorough knowledge of adult structure, and is shown to be worthless only when tested by the facts of embryology. According to this view the Brachiopoda and Polyzoa are connected with the Lamellibranchs through the Tunicates. The branchial sac of a Tunicate corresponds to the permanently retracted tentacular crown of the Polyzöon, and this again is homologous with the gills of a Lamellibranch. The recent great additions to our knowledge of the embryology of the Tunicata and Mollusca, and to our knowledge of the Lamellibranchiata gill, show with absolute conclusiveness that we here have nothing but an adult resemblance, reached in the two forms in entirely different ways, and without morphological significance.

Whatever view of the Vertebrate affinity of the Tunicata we may incline to, we must recognize the fact that the branchial sac is, morphologically, part of the digestive tract, and is in no sense whatever comparable with a lophophore or with a tentacular gill.

Moreover, we should expect, according to all analogy, to find the affinity to other groups most clearly shown by low or embryonic forms, but Appendicularia presents none of the characteristics upon which this comparison is based, and does not in the least resemble either the Lamellibranchs, Polyzoa or Brachiopods. This view is,

however, still maintained by many authors;<sup>1</sup> as an example, I may refer to Rolleston's *Forms of Animal Life*, ed. 1870, Introduction, pp. xxviii and lxxxvii, and Plate XI.

While most of the more recent writers recognize a pretty close relation between the Polyzoa and Brachiopoda, some associate them with the Mollusca, others regard them as a related group, (Molluscoida,) and others associate them with the Vermes. Huxley (*Anatomy of Invertebrated Animals*, p. 468,) says:

"The acceptance of the view originally propounded by Steenstrup, and so ably urged by Morse, respecting the affinities of the Brachiopoda with the Worms, does not, to my mind, weaken the opinion I have always held as to their affinities with the Polyzoa, on the one hand, and with the higher Mollusca, on the other."

On page 453, he says:

"The higher Mollusks, in fact, form the final term of a series of their own, which commences in the Polyzoa, with animals which have many resemblances to the Rotifera."

Claus takes a somewhat similar view, and places the Brachiopoda and Polyzoa together between the Annelids and Mollusca. He says, (*Grundzüge der Zoologie*, 1876, p. 821:)

"Die Brachiopoden hat man oft als nahe Verwandte der Lamelli-branchiaten betrachtet, die neueren Untersuchungen insbesondere über

<sup>1</sup> In a paper read Feb. 2, 1876, before the Boston Soc. Nat. Hist. on the Affinity of the Molluscoida, I inadvertently (page 5,) appeared to refer to Lankester, as one of the adherents to this view. It is hardly necessary to point out that this author was, on the contrary, one of the first to accept the view that the Tunicates are in no way related to the Mollusca or Molluscoida.

die Entwicklung haben jedoch gezeigt, dass unsere Thiere zu den Bryozoen und Anneliden in näherer Beziehung stehen. Vorläufig mögen sie als Anhang den Mollusken angereicht werden, denen sie unter Voraussetzung einiger wesentlicher Abänderungen und Vereinfachungen des Baues immerhin noch untergeordnet werden könnten. Jedenfalls würde die Bezeichnung Molluskoideen die nächste Berechtigung für die Brachiopoden haben. Dem gegenwärtigen Stande unserer Kenntniss würde es freilich am besten entsprechen, für Bryozoen und Brachiopoden einen besondern Typus als Molluskoideen aufzustellen, und denselben zwischen Würmer und Mollusken einzuschieben."

Gegenbauer (Grundriss der Verg. Anat., 1878, pp. 134 and 325,) separates the Polyzoa from the Brachiopoda, and places the former group among the Vermes, while of the latter he makes a primary group which, however, he places near the Annelids. In reference to the position of the Brachiopoda, he says:

"Früher meist den Mollusken beigezählt, mit denen sie wenig mehr als den Besitz einer vom Molluskengehäuse noch dazu ganz differenten Schale gemein haben, bilden die Brachiopoden eine kleine und eng abgegrenzte Abtheilung, die ihren Ursprung zum Stamme der Würmer zurückverfolgen lässt. Hier sind es die Chätopoden, also schon höher differenzierte Formen, bei denen sich manche Anschlüsse erkennen lassen, aber nur manche, denn gerade in den wichtigsten Organsystemen ergeben sich so bedeutende Eigenthümlichkeiten, dass es gewagt wäre, auf jene Beziehungen eine bestimmte phylogenetische Behauptung zu gründen. Jedenfalls ist der gesammte Organismus der Brachiopoden im Vergleiche mit Chätopoden und Anneliden total umgestaltet, und lässt nur noch in einzelnen Rudimenten seine verwandtschaftlichen Beziehungen wahrnehmen."

According to Lankester, the Brachiopoda and Polyzoa are closely related to each other, and are to be associated with the Mollusca, but his conception of the precise nature of this relationship is different from that of any of the other authors quoted. He does not, like Huxley, regard the

group Mollusca as a single series, with the Polyzoa at the bottom and the higher forms at the top, nor does he accept the division of the group into Mollusca and Molluscoida, but divides it into two *branches*, the Eucephala and the Lipocephala, and in the second branch he places the Polyzoa, Brachiopoda and Lamellibranchia. (Quart. Jour. Mic. Sc., Oct., 1877, p. 448.) Finally, we have the extreme view of Morse, that the Brachiopods are not merely related to the Vermes, but are actually ancient cephalized Chaetopod Annelids. In his paper upon the "Systematic Position of the Brachiopoda," (Proc. Boston Soc. Nat. Hist., XV, 1873,) he summarizes his views as follows, (p. 57:)

"In the Brachiopods, while in every feature of their internal structure betraying their annelidan affinities, and while the errantian forms with their long vermiform and annulated peduncle, their locomotion by means of setae, and their power of fabricating a sand tube, unite them clearly with the fixed and highly cephalized Chaetopods: yet in those groups that are attached, a remarkable concentration is seen, and many features are presented which have heretofore obscured the affinities of the group.

"To sum up the whole then. Ancient Chaetopod worms culminated in two parallel lines, on the one hand, in the Brachiopods, and on the other, in the fixed and highly cephalized Chaetopods. The divergence of the Brachiopods having been attained in more ancient times, a few degraded features are yet retained, whose relationships we find in the lower Vermes; while from their later divergence the fixed and cephalized Annelids are more closely allied to present free Chaetopods. And so we must regard the Brachiopods as *ancient cephalized Chaetopods*, while Serpula, Amphitrite, Sabella, Protula and others may be regarded as *modern* (later) *cephalized Chaetopods*."

It is rather difficult to decide what Morse's views are as to the relation of the Brachiopods to the Polyzoa. In the paper entitled "Early

Stages of Terebratulina," (1869,) he recognizes such a relationship, p. 36, and calls attention to many points of close resemblance between the embryo of Terebratulina and an adult Polyzöon.

In his second paper, on the "Systematic Position of the Brachiopoda," p. 47, he says:

"Naturalists are sufficiently well acquainted with the relations repeatedly pointed out, as existing between the Brachiopoda and the Polyzoa, and there is no necessity of again repeating them."

In his latest paper upon the subject, "The Embryology of Terebratulina," (1873,) he seems to be less inclined to accept this view, and on page 260, says:

"As to the relations of the Brachiopods with the Polyzoa, some features of similarity are seen between the embryo Brachiopod and the free embryo of Pedicellina, though the development of parts within a coenœcium and the formation of statoblasts are features quite unlike the Brachiopoda. A roundabout relation might possibly be insisted upon through the Rotifera, in their winter ova."

Packard, in his "Life Histories," 1876, gives a still different opinion. He says, p. 149:

"It will be seen that neither in the Polyzoa nor Brachiopods are there any true molluscan characters, nothing homologous with the foot, the shell-gland or odontophore. The Brachiopods should in our opinion be, perhaps, united with the Polyzoa and form a group lower but sub-parallel with the Annelides. The Brachiopods, from the facts afforded by Morse and others, have neither such a nervous system or respiratory or circulatory organs, or an annulated body, as would warrant their union with the Chaetopods. Morse has fully proved that they are a synthetic type, combining the features of different groups of worms, and this fact apparently forbids their being regarded as a group of Chaetopods.

"Looking at the subject from an evolutionary point of view, we should be inclined to regard the Brachiopods and Polyzoa as derived from common low vermian ancestors, while the Chaetopod worms probably sprang independantly from a higher ancestry."

These various selections are enough to show the wide divergence of views among modern writers upon the affinities of the Brachiopoda, and it is not necessary to extend the list, but no two authorities will be found to take precisely the same view, and our citations of divergent opinions might be continued almost indefinitely. This difficulty in determining the true position of the group, ceases to be surprising when we recollect that the Brachiopods have been in existence through almost the whole period during which we have any evidence of the existence of life, and that, of the numerous and varied forms which have existed in the greatest diversity during the past, only a few isolated forms are now available for study.

The difference of opinion will be found upon comparison, to be mainly upon three points: 1st. Are the Polyzoa and Brachiopoda so closely related as to form a natural *phylum*? 2d. Assuming that they are, are their affinities with the Vermes, or with the Mollusca? 3d. Assuming their relationship to the Mollusca, in what does it consist, and with what Mollusca are they to be classed? It is now an accepted law of morphology, that such questions are to be decided mainly by the study of embryology. That classification based upon adult structure is purely ideal, and may or may not express phylogenetic

relationship, and that "the study of the development of individual animals, is alone competent to decide whether this ideal unity has a foundation in observed fact." (Huxley, *Anat. of Invert. Animals*, p. 681.)

It would seem that our embryological knowledge ought now to be sufficiently comprehensive to enable us to give demonstrable solutions to these questions, for imperfect as our knowledge still is, we know something of the embryology of nearly all those families of Brachiopods which are now represented by living species, and there is, perhaps, no equally important group of animals regarding whose embryology our knowledge is more extensive, as compared with the number of existing representatives.

From the observations of Lacaze-Duthiers, (*Ann. d. Sc. Nat.*, xv, 1869, p. 259;) Morse, (*Embryology of Terebratulina*,) and Kowalevsky, (*Obs. upon the Development of the Brachiopoda*, 1874,)<sup>1</sup> we have a pretty complete history of the development of a number of genera of the hinged Brachiopoda or Testicardines. Lacaze-Duthiers and Kowalevsky having studied the embryology of *Thecidium*; Morse and Kowalevsky, *Terebratulina*, and Kowalevsky, *Terebratulina* and *Ar-*

<sup>1</sup> My acquaintance with the contents of the Russian paper, by Kowalevsky, which I have not seen, is through the very short abstract given by Claus, in his *Zoologie*.

giope. According to these observers, the egg of a hinged Brachiopod gives rise after segmentation and the formation of a primitive digestive cavity, to a ciliated elongated embryo, which soon becomes divided by constrictions into three segments. (See Figures 17 and 19, after Lacaze-Duthiers.) The digestive tract, 6, is a pouch which extends about as far backwards as the middle of the second segment, which we may call the body segment, 4. The anterior segment (3, Figures 17 and 19,) develops a circlet of long cilia, which, in some of the genera, at least, is an organ of locomotion.

Soon, in Thecidium and Terebratulina at least, the trochic segment divides into two, an anterior smaller one, Figures 17 and 19, 1, which is little more than a prominence upon the anterior face of the next segment, and which carries the mouth opening and also in Thecidium four pigmented eye spots. This segment we may call the oral. The circlet of cilia is now carried upon the second, much larger segment, which may therefore be termed the trochic segment, 3. Posterior to the body segment is a fourth small segment, 5, which does not contain any part of the digestive cavity. At a later stage of development, the embryo attaches itself by the posterior end of this segment, which then becomes converted into the peduncle; it may therefore be called the peduncular seg-



ment. The dorsal and ventral surfaces of the body segment now give rise to two folds, which become the two halves of the mantle; they gradually extend forwards and cover up the oral and trochic segments, and also extend backwards so as to cover the posterior portion of the body segment and part of the peduncular segment. The embryo attaches itself by its peduncular segment; the two mantle folds become covered by the two valves of the horny shell, and setae make their appearance around their edges; the anterior segments give rise to the brachial organs, and the segmented larva is thus converted directly into the attached Brachiopod.

In the hingeless Brachiopods, which are the least specialized and oldest forms, the process of development is much more complicated, and before the adult form is reached, the animal passes through a free-swimming stage, during which it attains to considerable specialization of structure, and becomes quite highly organized at a time when it lacks many of the most important characteristics of an adult Brachiopod.

The larva at this stage has no setae, no peduncle, and no indication of transverse segmentation. It is enclosed within a bivalve flattened shell, within which the digestive organs are loosely suspended in a body-cavity. The large stomach consists of two chambers, and from the posterior one the

intestine runs forwards or towards the mouth, to open ventrally, but to the right of the median line. The mouth opens in the centre of a somewhat horse-shoe shaped lophophore, which is capable of more or less extension or protension towards or beyond the anterior margin of the shell, and which is surrounded by a circlet of ciliated movable, non-retractile tentacles; the ends of the horse-shoe point towards the ventral surface, and the nerve-ganglion which first appears is between the mouth and the anus, which is outside the circle of tentacles. The body-cavity contains a corpusculated fluid which is kept in motion partly by ciliary action and in part by the motions of the body. The transformation of this larva into the adult is gradual and without any marked metamorphosis, and the larval stage is confined to the hingeless Brachiopods.

If, now, we compare these embryonic stages of the Brachiopoda with the various embryos and adults of the animal kingdom in order to find the forms to which they bear the greatest resemblance, we notice, first, the resemblance pointed out by Morse, (Systematic Position of the Brachiopoda, p. 43.) between the ciliated segmented embryo and the embryos of the many forms of invertebrate life which have been associated together as Vermes, but if we make a more detailed comparison. I think we must conclude that the par-

ticular form of ciliated embryo, which that of the Brachiopods is most like, is that of the marine Polyzoa.

From the study of the embryology of a large number of marine Polyzoa, Loxosoma, Pedicellina, Phalangella, Alcyonidium, Lepralia, Porella, Disuscopora, Mollia, Bicellaria, Scrupocellaria, Canda, Bugula, Vesicularia, Serialaria, Illustrella, Membranipora, and Eucratea, Barrois shows (*Mémoire sur l'Embryologie des Bryozoaires*,) that the various forms of polyzoan embryos, which appear at first sight to be widely dissimilar and independent of each other, can be shown to be modifications of one simple form, which form is, however, not represented by any existing embryo, and is purely ideal. This archetype is represented in Figure 7, Plate XVI, of his paper, and the figure is copied in outline as Figure 18, Plate 6, of the present paper. It is what Lankester (*Notes on Embryology and Classification*, Quart. Mic. Jour., Oct., 1877,) calls a "Telostomiate Architrochic" embryo, which, according to this author, is the hypothetical type of which the ciliated embryos of most of the invertebrates are modifications. It consists of a nearly cylindrical body, 4, 5, with a mouth, 2, at one end, which is surrounded by a circular band of cilia, 3. The mouth opens into a digestive cavity, 6, which is separated by a body-cavity from the body-wall.

Most of the polyzoan embryos, figured by Barrois, as so highly specialized, that the resemblance to the hypothetical archetype is not very apparent without careful comparison; but in others, the specialization is less marked, and the resemblance is much more obvious; and in *Loxosoma*, which is, in many respects, the least specialized of the Polyzoa, we have, according to the observations of Kowalevsky. (Mem. Acad., St. Petersburg, 1866;) Barrois, (loc. cit.) and Vogt, (Arch. Zoölogie Experimentale, V. 1876, trans. by Hincks, Quart. Mic. Jour., Oct., 1877.) an embryo which is readily seen to be a pretty direct modification of the ideal type. In Figure 20, Plate 6, I have copied in outline one of Barrois' figures of the embryo of *Loxosoma*. (Plate XVI. Figure 8.) for comparison with Figure 18. The general resemblance is very close, but the embryo is much more specialized than the type. The mouth, Figure 2, is situated in the centre of an eminence or oral segment, 1, the ventral margin of which is produced into a singular tongue-like structure which is covered with long hairs, and is probably a sensory organ. The architroch is carried upon a distinct segment, 3, which is sharply separated from those before and behind it. The posterior portion of the body is divided into a body segment, 4, in which the digestive cavity is suspended, and which carries a singular sen-

sory organ upon its ventral surface, and a tail segment, 5.

If, now, we compare this architrochic embryo of *Loxosoma* with the embryo of *Thecidium*, as figured by Lacaze-Duthiers, we shall find that there is not merely a general similarity, such as Morse has pointed out between the embryo of *Terebratulina* and those of the Annelids and Rotifera, but a very close and minute resemblance which extends even to details. In fact, almost the only differences between the embryo of *Loxosoma* and that of *Thecidium* are those which relate to the organs of special sense. In Figures 17 and 19, Plate 6, I have copied in outline Figures 9 and 10, Plate 5, of Lacaze-Duthiers' paper, and in Figure 21 another (Plate I, Figure 14,) of Barrois' figures of the *Loxosoma* embryo. In each we have a bilaterally symmetrical embryo which is divided into four segments.

The most anterior segment, 1, is small in both and carries the mouth and special sense organs. The second or trochic segment, 2, is much larger, and its margin is set with long locomotor cilia. The digestive organs, 6, in each are loosely suspended in the body-cavity and reach about as far backward as the middle of the third or body segment, 4, and the fourth segment, 5, is much smaller than the third, and substantially alike in both. In both the dorsal surface is convex

and longer than the nearly straight ventral surface, and a comparison of Morse's Figure 87, Plate IX. (Embryology of Terebratulina,) with Barrois' Figures 17, 18 and 20, Plate I, will show that the side view of the Brachiopod embryo is very similar to that of the *Loxosoma* embryo. The similarity of the embryo of *Loxosoma* to the archetype and the very unspecialized character of the adult, are very strong grounds for regarding its embryo as a very slightly, if at all "falsified" ancestral form, the "stock" from which the Polyzoa are descended, and it seems clear that the resemblance between this and the segmented embryo of the Brachiopods is much more perfect and detailed than the resemblances shown by Morse to exist, in a very general way, between the Brachiopod embryo and the embryos of Annelids, Gephyreans and Rotifera.

So far as this stage of development is concerned, then, I think we are now in a position to affirm and prove that the Brachiopod is more like the unspecialized polyzoan embryo, than it is like any other known organism, embryonic or adult.

Passing now to the free larva of the hingeless Brachiopods, we cannot fail to notice the great resemblance which it bears to the adult Polyzoa; especially to the incrusting forms and to the hippocrepian fresh water forms. Although Morse (Systematic Position of the Brachiopoda, p. 44.)

says of Müller's paper: "What could be more annelidan than the description of this larva?" Müller himself does not speak of any marked resemblance to an Annelid, and notices only the similarity to a Polyzöon, and McCrady notices the same resemblance. Our account of the organization of the *Lingula* larva shows that it is not only like a Polyzöon, but that it actually is one; as much so as the hydra stage of an Hydro-Medusa is a Hydra, or the tailed larva of Botryllus is an Appendicularia, and more so than a Tadpole is an Urodellan Batrachian.

If it were not a larva, but an adult, it would unquestionably be regarded as a solitary, swimming Polyzöon, with a highly specialized nervous system and sense organs. It resembles the in-crusting Polyzoa in the possession of a bivalve, movable shell, and it resembles the fresh water Polyzoa in the form of its lophophore, and it differs from all the Polyzoa in being solitary, and in the presence of peculiar muscles, and in the perfection of its nervous system, and it cannot therefore be placed in any of the existing families of Polyzoa, but if it were an adult, it would not demand a new class for its reception, for it differs from ordinary Polyzoa much less than Rhabdopleura does.

The study of the summary given on pages 87 and 88, and of the section shown in Figure 16, Plate 6, will show that the resemblance to a

Polyzöon is both general and minute, and it does not seem necessary to point out the points of agreement.

Now what interpretation are we to give to these polyzoan resemblances? Morse has shown that the adult Brachiopod resembles the Annelids in many particulars, such as the presence of segmental organs which connect the body-cavity with the exterior, the possession of chitinous setae, and of a vascular system which is distinct from the perivisceral circulation, and which is filled with the pseud-haemal fluid distinct from the corpusculated nutritive fluid which fills the body-cavity, but we now know that these annelidan resemblances are reached, at least in the hingeless forms, by the specialization of a polyzoan larva, which is annelidan in no sense whatever.

The annelidan resemblances of the adult Brachiopod are unquestionable, and so also are the polyzoan resemblances of the larva, and we may suggest two methods of interpreting the facts. We may regard the development of the hinged Brachiopods as the ancestral, unmodified form; the resemblance of the adult to an Annelid as the evidence of descent from an annelidan ancestor; the polyzoan larva of the hinged forms as a "falsification" of the embryological recapitulation of the ontogenetic process, and the resemblance



to a polyzoan as an acquired adaption which is not due to descent; or, on the other hand, we may regard the development of the hingeless Brachiopoda as the true ontogenetic record, and the direct conversion of the segmented embryo into the adult, in the hinged genera, as a synco-pation of the record by acceleration of the time of acquisition by the young of the characteristics of the adult. In this case we must regard the resemblances of the adult Brachiopod to an Annelid as acquired adaptations, without morphological significance, and of the same character as the resemblance of an adult Tunicate to a Lamellibranch.

The association of the Brachiopods with the Annelids, on account of these resemblances would then be exactly comparable, so far as scientific value is concerned, with the old view that they are joined to the Lamellibranchs through the Polyzoa and Tunicata, and we should be compelled to search for evidence of the affinity of the Brachiopoda to other invertebrates, in the structure of the Polyzoa, and that of the segmented architrochic larva, but neither of these presents features upon which an affinity to the Annelids, as distinguished from many other invertebrates, could be founded.

As we can study the embryology of the existing species only, we cannot have any direct proof

of either of these alternatives, and must decide between them from the analogy afforded by the embryology of other groups of animals.

It is a well known embryological law, proved by observation, and capable of deductive demonstration, that the higher or more specialized forms of a group, frequently show a more or less marked tendency to jump some of the embryonic stages through which the lower forms pass. They all start alike, but the time of appearance of the adult characteristics, is "accelerated" in the higher forms, and the development thus simplified. The Cephalopoda among the Mollusca; certain Decapods among the Crustacea, and Salpa among the Tunicates, are well known examples.

As the hinged Brachiopods are much more specialized than the hingeless; the analogy of other groups would seem to indicate that the larval stage of the latter is ancestral, and the direct development of the hinged forms a secondary effect; but there is also an opportunity to cite analogy in support of the other view: that the larval stage of the hinged forms is an adaptation.

The chrysalis state of the Lepidoptera and other insects is a well-known instance of such acquisition of embryonic characteristics. While we should not expect the somewhat locomotive adult *Lingula* to have acquired a locomotive larval stage which has not been acquired by the entirely stationary

Terebratulina, we cannot deny the possibility of this, and we must test the question by a comparison of the larva with a Polyzöon, in order to decide whether the resemblances between them are superficial and such as we should expect to be brought about by similar conditions, or whether they are deep seated and of morphological value. We must compare the resemblances between the adult and an Annelid with those between the larva and a Polyzöon, in order to see which furnish the best proof of genetic relationship; which set of resemblances are latest, and grow most marked as the *Lingula* approaches maturity, and which are most embryonic and disappear as the specializations of the adult are acquired. As Morse is the most able advocate of the relationship of the adult to an Annelid, we may confine our attention to his statement of the case. In his summary, already quoted, p. 82, he lays especial stress upon the following resemblances of *Lingula* to a tubicolous Annelid; that it has a vermiform annulated peduncle; moves by setae, and makes a sand tube. The first of these resemblances is not real, as the peduncle of *Lingula* is not divided into segments; and the other two are certainly not features upon which it is safe to found a classification, for they are external adjustments to the environment, and precisely the sort of resemblances which are most easily brought about by adaptation.

On page 58, he gives in parallel columns, the following points of resemblance to the Vermes in general:

1. Vermes: Bilaterally symmetrical, depressed, flattened or circular, never flattened laterally.  
Brachiopods: Same.
2. Vermes: Above and below similar, or distinguished with difficulty.  
Brachiopods: Same.
3. Vermes: Most free, some attached.  
Brachiopods: Same.
4. Vermes: Muscles connected with integument, dorsally, ventrally and laterally.  
Brachiopods: Same.
5. Vermes: Two layers of muscles in body-wall.  
Brachiopods: Same.
6. Vermes: Digestive canal straight or seldom convoluted.  
Brachiopods: Same.
7. Vermes: Suspended in perivisceral cavity by bands of delicate tissue.  
Brachiopods: Same.
8. Vermes: Peculiar depuratory organs, consisting of bilaterally symmetrical tubes, opening externally, and communicating with the perivisceral cavity by ciliated infundibuliform orifices.  
Brachiopods: Same.
9. Vermes: Nervous system a simple oesophageal collar, with accessory ganglia, usually forming a chain.  
Brachiopods: Nervous system a simple collar.
10. Vermes: Generative products in most set free in perivisceral cavity.  
Brachiopods: Same.
11. Vermes: Possessing chitinous outgrowths, either as scales, or plates, or hairs, or spines, the latter being secreted by setigerous follicles.  
Brachiopods: Same.
12. Vermes: Cuticle perforated by minute pores.  
Brachiopods: Same.
13. Vermes: Perivisceral cavity ciliated in some cases.  
Brachiopods: Same in all cases.
14. Vermes: An extensive vascular system, containing a colored fluid, representing the pseud-haemal system. The corpusculated nutritive true blood is usually contained in the perivisceral cavity alone.  
Brachiopods: Same.
15. Vermes: Embryo distinctly transversely segmented.  
Brachiopods: Same.
16. Vermes: In some groups intestine having no anal outlet.  
Brachiopods: Same.

This is certainly a remarkable series of highly interesting resemblances, and is worthy of careful examination, but I think it proves much more and much less than the writer deduces from it.

In the first place, it will be noticed that the points given are not characteristics of the Annelids, but of the so-called "Class Vermes," and most naturalists are now agreed that the division Vermes is in no sense a natural group equivalent to such groups as the Mollusca, Echinoderms, or Annelids, but is a collection of numerous widely divergent forms of life, most of which are represented by a comparatively small number of subordinate groups, and accordingly have not been recognized as primary groups of equal morphological importance with those types which are represented by numerous modifications.

In pointing out that these various types of animal life, including the Brachiopods, have many features in common, Morse has done good service, and may claim to be a pioneer in that field of invertebrate morphology which has made the most important advances within the five years since the publication of his paper.

This period has been especially marked by the comparisons and generalizations which have been made from the mass of embryological and anatomical knowledge which had been accumulated during previous years. As the result of this com-

parison, we now know that not only the "Vermes" and Brachiopods, but the Echinoderms, Mollusca, Vertebrates, in fact all the Coelomatus Metazoa, have so many points in common as to indicate that they are all branches which diverged, very early, from a common bilaterally symmetrical worm-like form, but I do not think that the points above quoted, justify us in regarding the Brachiopods as more intimately associated with the "Vermes" than other groups, such as the Echinoderms and Mollusca.

Of the points quoted the 1st, 2d, 3d, 11th and 12th are plainly superficial and of little morphological value. Prof. Morse appears to attach great importance to the 11th, but even if chitinous setae could be shown to be confined to the Chaetopod worms and Brachiopods, which is certainly not the case, this fact in itself would not be of any greater importance than the fact that calcareous spicules are found in the Sponges, Echinoderms and some Nudibranchs. The 4th, 5th, 6th, 7th, 10th, 13th and 16th are all of them points which are not at all generally true of the so-called "Vermes," and none of them are confined to the animals which have been included in that group; for instance, the 4th, 5th, 6th, 7th, 13th and 16th hold true of certain Echinoderms, and the 4th, 5th, 6th, 7th and 13th apply to the Holothurians; most of them are also true of some Mollusca, and nearly all of

them are true of most Tunicates. With regard to the 9th point, the nervous system of an Echinoderm consists of a single œsophageal nerve ring, with accessory fibres; and we now know from the investigations of Von Jhering that the molluscan nervous system is fundamentally like that of an Annelid. The 10th peculiarity is not at all uncommon, and is true of most Vertebrates for instance. There remain then only the 8th and 14th points upon which to base the claim to a peculiar and especial affinity between the Brachiopoda and the Vermes. The resemblance in these particulars is undeniable and of the greatest interest, and five years ago was almost enough to establish Morse's view, but one of the most remarkable recent contributions to morphology—the result of numerous investigations by different observers—is the proof that the depuratory organs of nearly all of the Coelomatus Metazoa are fundamentally alike.

To quote from Lankester (Notes on Embryology and Classification:)

"In Rotifera, Flat Worms, Gephyraea, Mollusca, in the metameræ of Chaetopoda, in the Vertebrata, and even in some Arthropoda, we have evidence of the existence of a single pair of canals, more or less highly modified by glandular developments, which usually open by ciliated funnel-like mouths into the cœlom at one end and directly to the exterior in the neighborhood of the anus, or into a cloacal chamber, at their other end, thus placing the cœlom in communication with the exterior. This pair of ciliated funnels appears to be the same organ in all cases."

It is plain then that at the present time the presence of these organs in the Brachiopod cannot

be held to show any peculiar affinity to the "Vermes."

There remains then only the 14th point, and it is only necessary to call attention to the presence in the Echinoderms of a corpusculated perivisceral fluid, and a pseud-haemal system restricted to vessels, in order to show that this resemblance is too general to indicate the supposed close relationship. The segmented embryo I have already spoken of. The Brachiopods then are "Vermes" in the same sense that the Echinoderms, Mollusca, Tunicates and Vertebrates are, and in order to include them we must make the word almost synonymous with "Coelomatous Metazoa."

In the case of the Brachiopods and Polyzoa on the other hand we have a resemblance which is definite and minute; which is most marked during the youngest stage of development, and gradually becomes less conspicuous as the adult characteristics are acquired, and there appears to be every reason to conclude that the relation of the Brachiopoda to other invertebrates must be traced only through the Polyzoa.

Accepting then the relationship of the Polyzoa and Brachiopoda as proved, and recognizing the fact that the former is the ancestral form of which the latter is a specialization, we come now to the question: to what other forms are the Polyzoa most nearly related? There is certainly a great



general resemblance between a hippocrepian Polyzöon and a Rotifer, but there is also a fundamental difference in certain highly important particulars; for instance, the nervous system and bend of the tentacular crown are between the mouth and the anus in the Polyzöon, and on the side opposite the mouth in the Rotifer. In the former, the mouth is inside the crown of tentacles, and the latter is therefore "architrochic," while it is "cephalotrochic" in the latter, or with the mouth outside the circle. The resemblance between the two is very close however, and should find expression in a system of classification; but I think that the resemblance between a Polyzöon and the molluscan Veliger, is much more detailed and complete, and the relationship closer. In a paper upon the affinity of the Mollusca and Molluscoida, (Boston Soc. Nat. Hist., Feb. 2, 1876,) I attempted to show that the various groups of true Mollusca are related to each other, not through the Lamellibranch, but through the Veliger; and then gave the following statement of the affinity of the Veliger; a statement which I still regard as the true view:

Most of the Gasteropoda are known to pass through a free, locomotive "Veliger" stage. The Veligers of different Gasteropods differ considerably in form; and in some, the embryo at this stage, is much less specialized than in others; but, omitting the complications introduced as adaptations to a spiral shell, the Veliger of such a marine Gasteropod as *Astya*, may be regarded as presenting the typical form. A Veliger may be described as a free-swimming, bilaterally symmetrical embryo,

without a true heart or vascular system, or branchiæ, with the mouth and anus near each other on the median line. The digestive organs are suspended in the body-cavity, and attached to the body-wall at the two external apertures, and by the various muscles. The foot is situated between these two openings; and the pedal ganglia, which are in most Veligers the first ganglia to appear, are developed in the region of the foot; that is, between the mouth and the anus. The foot is generally supplied with a bunch of setæ, which are apparently sensory in function. The animal is enclosed in a shell composed of two portions; a large ventral cup, and a neural or pedal operculum, which is united to the anal margin of the cup at the earliest stages, and subsequently becomes separated from it. This shell and lid are found in the embryos of those forms where the adult is without an operculum, as *Crepidula*, as well as in those where the adult is destitute of a shell, as the Nudibranchs.

The most characteristic peculiarity of the Veliger is the *velum*. This is a large bilaterally symmetrical circlet of cilia, developed from the cephalic region of the embryo, and supported, at some distance from the body, by a transparent double-walled veil, the cavity of which is irregularly divided into large sinuses, in free communication with the body-cavity. The animal swims, usually near the surface of the ocean, by means of the long cilia of the velum, which would seem to perform the function of a respiratory organ as well, for the fluid which fills the body-cavity is driven into and out of the sinuses of the velum by the retraction and expansion of this structure; in most Veligers this circulation seems to be aided by the rythmical contraction of the muscular fibres which bind the foot to the œsophagus. The mouth is not within the circlet of large locomotive cilia, but immediately behind it, and a ring or band of smaller cilia passes from the anterior margin of the mouth entirely around the velum, on its lower surface, and therefore outside the circlet of locomotive cilia.<sup>1</sup> This second circlet seems adapted to convey food to the mouth, but there are no direct observations upon this point. The velum and the foot are retracted into the shell by the action of a pair of long muscles which pass from the sides of the œsophagus and region of the foot to the bottom of the ventral shell, and subsequently become the columellar muscle of the adult.

The conclusion to be drawn from our present knowledge of the Mollusca, will appear to be that all of them are to be traced back to a free-swimming ancestral form, of which the Veliger embryo is the representative; this seems to be the only way in which we can account for its appearance in at least certain representatives of so many widely separated groups.

We come now to the interesting question: what are the affinities of this "Veliger" from which the true Mollusca are descended?

<sup>1</sup> Since the paper quoted was written, I have observed this second band of cilia, which passes behind the mouth, in a number of Gasteropod Veligers.

It is only necessary to glance at the side view of any fully developed Veliger, such as Selenka's figure of *Tergipes*, in order to notice the resemblance to a Polyzöon, and more careful examination shows that the resemblance holds not only in the general plan but in detail. The velum corresponds to the lophophore in position and structure, and subserves, like this, the function of respiration, and probably that of ingestion as well. The heart is absent in both, and the fluid which fills the body-cavity and bathes the digestive organs is kept in motion by the contraction of the various muscles of the body. The digestive organs are similar in form and also in their connections. The epistome with its ganglion answers to the foot and pedal ganglia, and in *Rhabdopleura* the epistome is functionally as well as morphologically a creeping disc. The shell and operculum answer to the cell and lid of a cheilostomatous Polyzöon, and the retractor muscles are clearly homologous. The most important differences seem to be that among the Polyzöa, the animals are fixed and multiply by budding; and that in all, the mouth, as well as the epistome, is within the cirlet of the lophophore. (*Rhabdopleura* was described by Allman as an exception in this respect: Sars, however, has shown that although the tentacle-bearing portion comes to an end upon the sides of the foot, the line of cilia is continued entirely around it.) The lack of agreement between the positions occupied by the mouth and foot in the two forms seems to be the most serious objection which can be urged against the view here advocated. In answer to it we can only point out that in *Dentalium* the mouth is formed within the cirlet, although the foot is outside it. It is not to be supposed, however, that the Veliger can be traced back to any existing form of Polyzöon, or even to any order of this class. In some respects its affinities are with the Hippocrepia, in others they are with the Cheilostomata, and in still others they are with *Rhabdopleura*, and they therefore indicate that the common ancestral type of the Mollusca was not a true Polyzöon, but simply a Polyzöon-like form. A lack of agreement in points of detail is therefore no more than we should anticipate. In answer to the second objection, that the Polyzöa multiply by budding, we may refer to the well known law, that agamic vegetative multiplication is antagonistic to high evolution, and is accordingly replaced by true sexual reproduction in the higher forms of all classes of animals; as its presence, if it occurred in any of the true Mollusca, could not be regarded as proof of an affinity to the Polyzöa, its absence does not disprove such affinity. No one will attach much importance to the remaining objection, that the Polyzöa are fixed.

The similarity between the Polyzöa and true Mollusca, in general plan of structure, has long been recognized, but the attempts to connect the two groups through the Lamellibranchs are so evidently incorrect that, led by the unquestionable affinity of the Polyzöa and Brachiopods to the Vermes, many geologists are now inclined to separate these lower forms from the Mollusca proper. As soon as we recognize that the Lamellibranchs are not to be regarded as typical Mollusca, and that all of the

latter are to be traced back to a "Veliger" all difficulty seems to disappear, and it becomes plain, not only that Mollusca and Molluscoida are related, but that they are connected so closely, that the advisability of such a division is very doubtful. We also obtain, at the same time, an explanation of the worm-like early stages of the embryo, exhibited by so many of the true Mollusca. The belief so firmly supported by nearly all zoologists a few years since, that the various branches of the animal kingdom are absolutely independent of each other, has been almost entirely overthrown by the accumulation of new facts, and the constantly increasing tendency to examine them in their bearing upon the theory of the evolution of life; and the union or junction of the Vermes and the Mollusca, in some manner, has already found a number of advocates.

Prof. Morée, by his investigations upon the anatomy and embryology of the Brachiopods, has shown that, if we consider this group by itself, it must be placed with the Annelida. His investigations also show, with equal clearness, that the Brachiopoda are closely related to the Polyzoa, and we must therefore regard them as united by the "Veliger" to the true Mollusca. If we accept the view that the molluscan and vermian stems are thus united, the question,—“Are the Brachiopods Worms or Mollusks?”—will be regarded as nothing but a verbal discussion; for this class forms the connecting link between the two groups, and any sharp line of demarcation does not exist.

In the above paper, I proposed a provisional phylogeny of the Mollusca, which I should now accept only after considerable modification.

The Rotifera, Polyzoa and Veliger, seem to be three branches which diverged, very early, from a common vermian stem. The Brachiopoda are the most highly specialized representatives of the Polyzöon branch, and the true Mollusca stand in a similar relation to the Veliger branch. The three stems appear to be sufficiently closely related to each other, and sufficiently sharply distinguished from all other animals to constitute by themselves one of the fundamental divisions

of the animal kingdom, which might be called, on account of the conspicuous character of the trochal disk, the Trochifera.

In conclusion, I may be allowed to call attention to the obvious fact, that the persistence of *Lingula*, entirely without change, for a period which is too great to be measured in the terms of such a unit as the length of human experience, is a very forcible instance to show that the facts of zoölogy absolutely forbid the acceptance of any theory of necessary evolution by continuous progress from homogeneity to heterogeneity.

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#### EXPLANATION OF THE FIGURES.

With the exception of Figure 16, the letters of reference have the same significance throughout, but this is not true of the numbers.

- A.* Dorsal or aboral valve of shell.
- B.* Ventral or oral valve.
- C.* Peduncular or posterior end of shell.
- D.* Anterior end.
- a, a.* Hinge-teeth of aboral valve.
- b, b.* Hinge-teeth of oral valve.
- c.* Semicircular plate of aboral valve.
- d.* Median or aboral tentacle.
- d<sup>1</sup>, d<sup>1</sup>.* The pair of oral tentacles.
- d<sup>2</sup>, d<sup>2</sup>, d<sup>3</sup>, d<sup>3</sup>, &c.* The tentacles of one side numbered according to their order of appearance.
- d<sup>n</sup>.* The most recent pair of tentacles, in process of development at the sides of the aboral tentacle.

- e. The lip, formed by a crescent-shaped fold of the oral surface of the lophophore.
- f. Lateral walls of the body, (*parietal bands*.)
- g. Body-cavity.
- h. Liver.
- i. Oesophagus.
- k. Hepatic chamber of stomach; the "cardiac" chamber of the Polyzoa.
- l. Intestinal chamber of stomach; the "pyloric" chamber of the Polyzoa.
- m. Intestine.
- n. Anus.
- o. Mouth.
- p. Muscles which pass from one valve to the other on each side of the oesophagus near its union to the stomach; they occupy the same position as those muscles of the adult which in the figures (after King) of the article "Brachiopoda" in the *Encyclopædia Britannica* (ninth ed., vol. 4, p. 193, Figs. 23, 24 and 25.) are lettered *AA*; but during the larval stages they probably resemble not these muscles alone, but also those lettered *jj*, *kk* and *ll* in the above article.
- q. q. The lophophore.
- r. Posterior unpaired muscle. (*g* in the article above referred to.)
- s. Peduncle.
- s'. Free end of peduncle.
- s''. Cavity of peduncle.
- t. Palatal cavity.
- u. Ventral end of the muscle, *j*, of the article "Brachiopoda."
- u''. Dorsal end of the muscle, *i*, of the same article.
- u'. Dorsal end of a muscle which probably represents the adult muscles *c* and *d* in the same article.
- v. Palatal sinus.
- w. Its opening into the body-cavity.
- x. Ventral portion of nerve ring.
- x'. Oesophageal commissure.

- x''*. Ganglionic enlargement of *x'*.  
*x'''*. Otocyst.  
*y*. Retractor muscles.  
*z*. Reflection of dorsal portion of the body-wall onto the inner surface of the shell.

## PLATE I.

**FIGURE 1.**—Dorsal view of the youngest larva which was figured, magnified 250 diameters, (Zeiss Obj. D.; Eye-piece 2.) The vase-shaped body, containing the digestive organs, occupies the middle line, and the integument adheres closely to the surface of the stomach and liver, thus almost obliterating the body-cavity. At its upper end the œsophagus bends towards the ventral surface, and the mouth, *o*, is seen through its transparent wall. The lophophore carries five pairs of tentacles, besides the single median dorsal tentacle, *d*. Posterior to the mouth the lophophore is below or ventral to the œsophagus. Above the mouth the lip, *e*, is seen through the transparent lophophore. The pallial space is a capacious chamber, *t, t, t*, on the sides of and in front of the animal.

**FIGURE 2.**—A little older larva, magnified 250 diameters, and seen from the ventral side. The intestine, *m*, is seen opening into the pallial chamber on the right side of the body. The integument is separated from the surface of the digestive organs, by a distinct body-cavity, *g*. On the right side of the posterior end of the body, the peduncle, *s*, has appeared. The ciliated mouth, *o*, and lip, *e*, are here shown in surface view in the centre of the lophophore, which now carries eight pairs of tentacles on each side.

## PLATE II.

**FIGURE 3.**—Dorsal view of a somewhat older embryo, seen with the same magnifying power. The drawing has

been somewhat reduced, but as the line, *aa*, has the same absolute length in all the larvae, the increase in size may be seen by comparing this line in Figures 2 and 3. The shell has elongated, and the thickened margin of the mantle has begun to develop setae. The peduncle has increased greatly in length, and is folded across the posterior end of the body, and does not project from the shell. The body-cavity is now quite conspicuous, and the integument is quite widely separated from the surface of the digestive organs. The nerve ring and otocysts are now seen around the anterior end of the stomach; the number of tentacles has increased to ten pairs, and the sides of the lophophore begin to fold towards the dorsal surface.

FIGURE 4.—Dorsal view of the body of the larva shown in Figure 2, somewhat compressed and magnified 740 diameters, (Zeiss, D. 4.) The stomach, *k*, *l*, and liver, *A*, have been somewhat flattened by pressure, thus obliterating the body-cavity, which should be visible between the surfaces of these organs and the integument, *f*. The dorsal ends of the adductor muscles, *p*, have also been pushed over the oesophagus by the pressure. The otocysts, *x''*, the lateral ganglia, *x''*, and the ventral ganglion (seen through the oesophagus,) are shown, but the commissure, *x'*, does not yet encircle the digestive tract.

#### PLATE III

FIGURE 5.—Ventral view of the body of a larva of about the same age as that shown in Figure 3. The ventral nerve mass, *x*, is shown upon the oesophagus. The integument is widely separated from the digestive organs, and the ciliated body-cavity is filled with blood corpuscles, as is also the ciliated cavity of the peduncle. From the anterior end of the ventral surface of the body, two long horn-like diver-



ticula from the body-cavity penetrate the mantle of the ventral valve, and run forwards, forming the pallial sinuses, *r*. They are ciliated, filled with blood corpuscles, and communicate with the perivisceral space by large funnel-shaped ciliated apertures, *w*.

FIGURE 6.—Dorsal view of the same larva. The nerve ring, *x'*, the lateral ganglion, *x''*, and the otocysts, *x'''*, are shown between the integument and the digestive tract; and the integument bulges outwards on each side, over the lateral portions of the nervous system.

#### PLATE IV.

FIGURE 7.—Ventral view of a small *Lingula* soon after it becomes sedentary; the actual length of the shell is shown by the short line at the right; and the relative size as compared with Figure 3, by the line, *nu*, the edge of the larval shell. The tentacles are now 16 in number on each side, and the lateral margins of the lophophore begin to bend towards the dorsal surface. At this stage the shell was too opaque to allow of the minute examination of the digestive organs, which are only shown in outline.

#### PLATE V.

FIGURE 8.—The same embryo in a dorsal view, with the dorsal valve, *B*, displaced by rotation upon the body. The bending of the lateral edges of the lophophore is well shown at *q, q*.

#### PLATE VI.

FIGURE 9.—Dorsal view of the posterior end of the body of an embryo a little older than Figure 1, to show the relation of the peduncle to the body-wall; greatly magnified.

FIGURE 10.—Ventral view of the same embryo.

FIGURE 11.—Ventral view of the same organs at the stage shown in Figure 2.

FIGURE 12.—Blood corpuscles of adult *Lingula*.

FIGURE 13.—A portion of the lophophore.

FIGURE 14.—A single tentacle, more highly magnified.

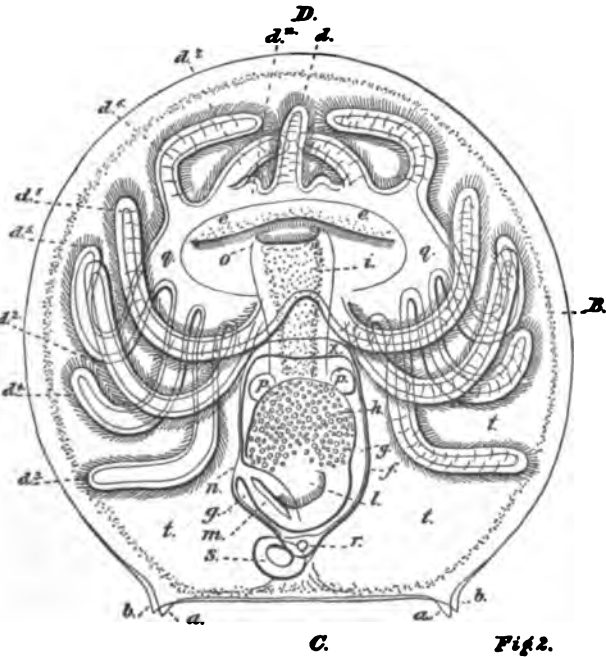
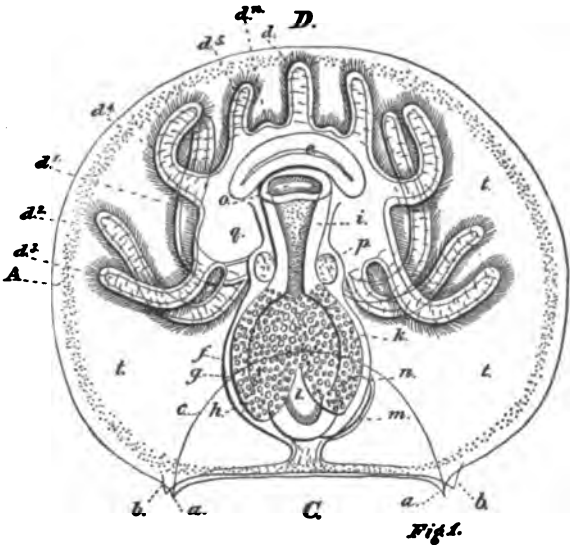
FIGURE 15.—Proximal ends of three tentacles.

FIGURE 16.—Diagram of longitudinal section of the embryo, at about the stage shown in Plate 2, Figure 3.

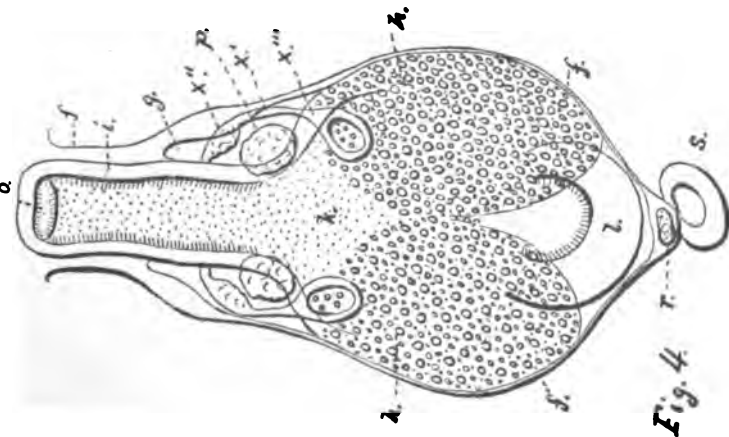
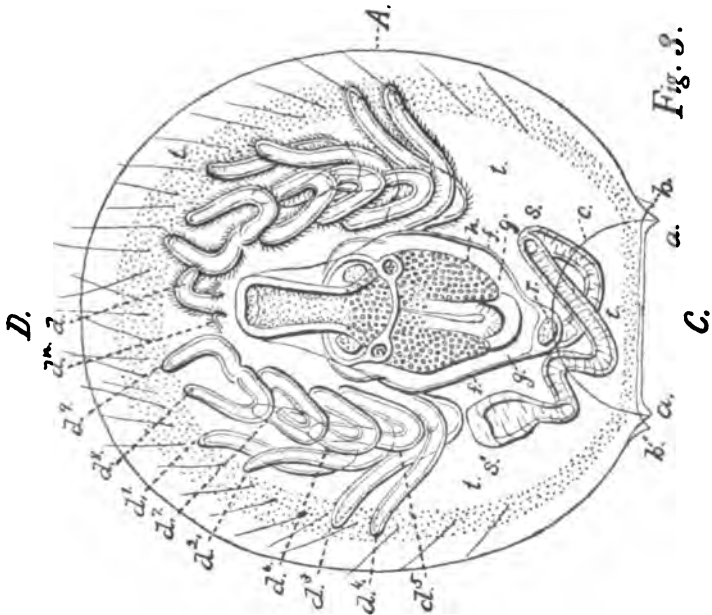
- a. Tips of valves.
- b. Thickened margin of mantle.
- c. Mantle.
- d. Dorsal median tentacle.
- e. Lophophore.
- f. Lip.
- g. Mouth.
- h. Mouth cavity.
- i. Body-cavity.
- k. Wall of œsophagus.
- l. Oesophagus.
- m. Hepatic chamber of stomach.
- n. Intestinal chamber of stomach.
- o. Intestine.
- q. Ventral ganglion.
- r. Posterior muscle.
- s. Dorsal valve of shell.
- t. Ventral valve of shell.

FIGURES 17 and 19.—Embryo of *Thecidium*, from Lacaze-Duthiers

FIGURES 18, 20 and 21.—*Polyzoon* embryos, from Barrois.







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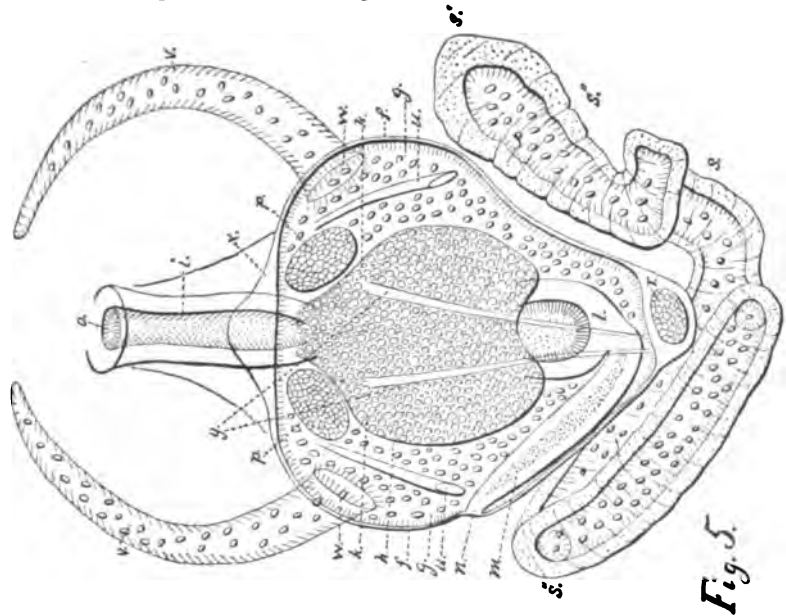


Fig. 5.

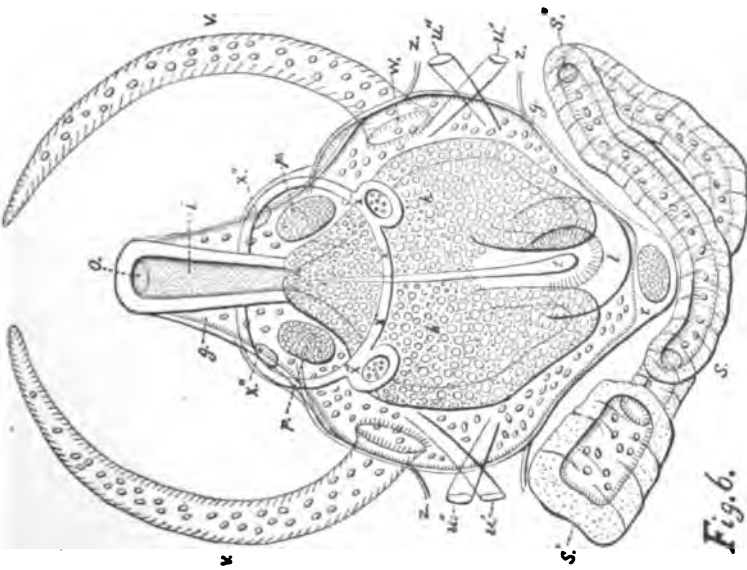
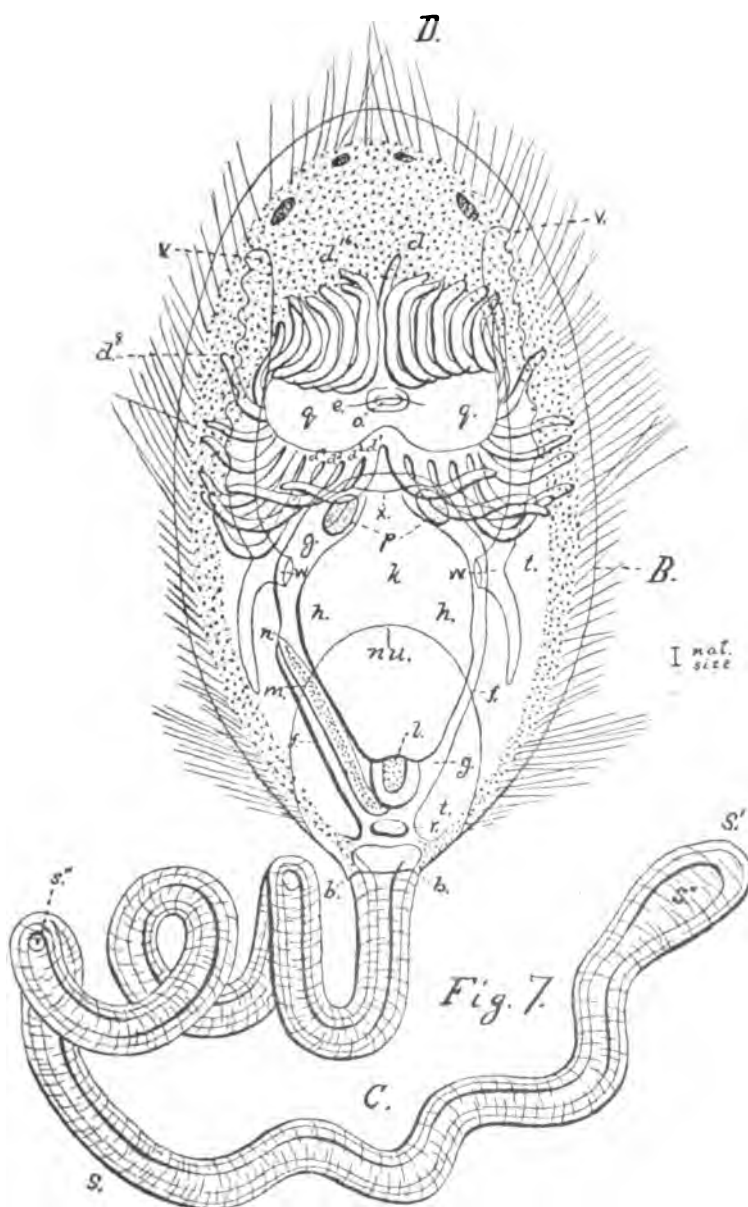


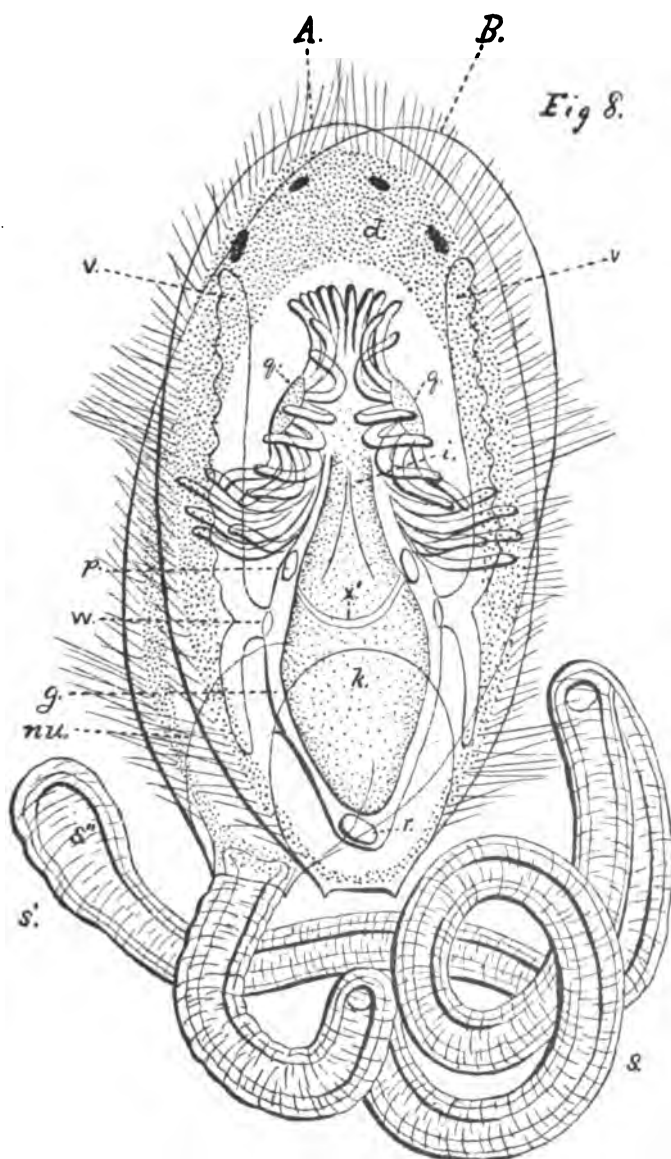
Fig. 6.







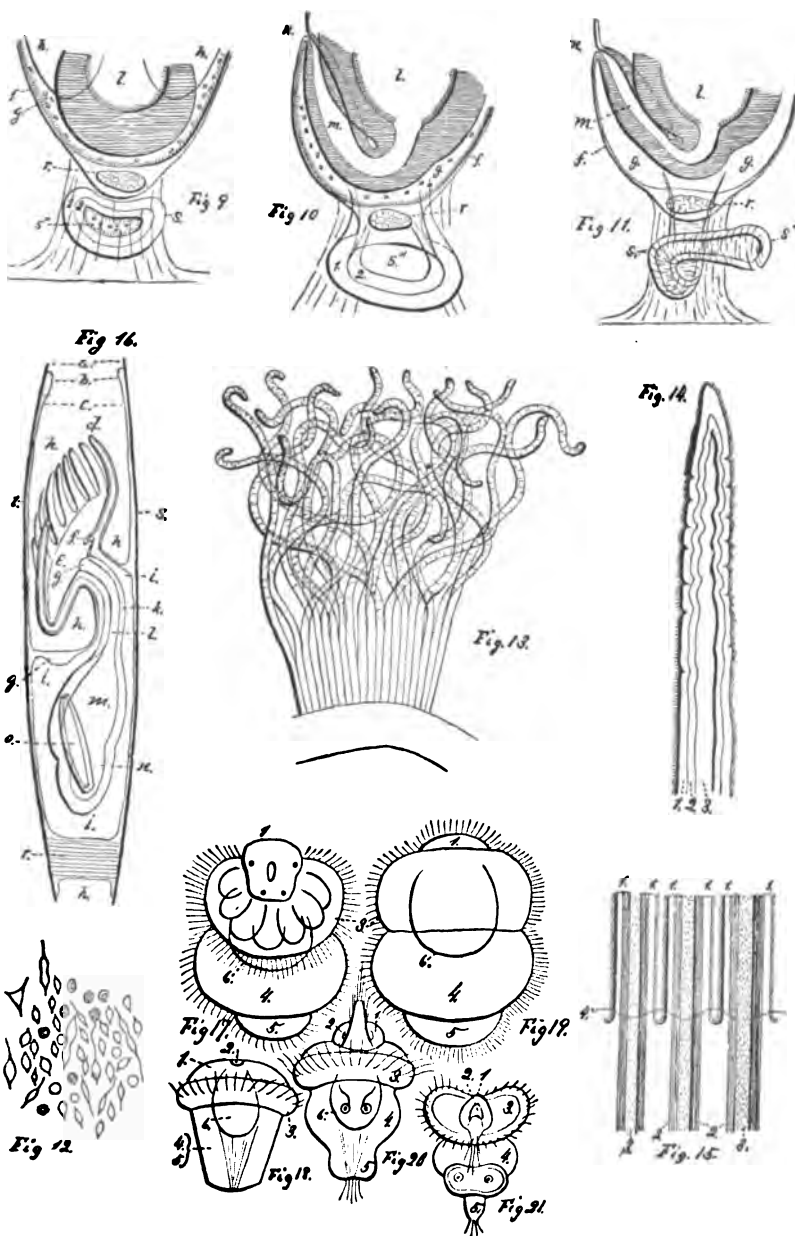






# *Development of Lingula.*

# *Plate 6.*



W. E. BROOKS, DEL.

1

## Description of Lucifer Typus. M. Edw.?

BY WALTER FAXON, *Museum of Comp. Zoölogy of  
Harvard College.*

DURING the early part of August a few specimens of the genus *Lucifer* were taken at night with the hand net, at the surface of the water, in the vicinity of Fort Wool, by Mr. August Schmidt.

As far as I know, this is the first record of the occurrence of this interesting genus on our shores. Messrs. Smith and Harger took a few specimens (species undescribed and undetermined) east of George's Bank, Lat.  $41^{\circ} 25' N.$ , Long.  $65^{\circ} 5' \text{ to } 30' W.$ , (Note 1.) The described species have come from various points in the Mediterranean, Atlantic, Pacific and Indian Oceans.

### DESCRIPTION.

Antennary segment twice as long as the carapace. A small spine (Figure 1, *s*,) projects from its anterior margin at the base of the eye-stalks.

There is no clear line of demarcation between the antennary segment and the carapace.

Carapace about as long as the first abdominal segment. Its inferior borders crenate. A minute spine on each side. (Figure 1, ζ.)

The first five segments of the abdomen are about equal in size, their latero-inferior margins produced into an obtuse angle at the middle. The sixth segment of the abdomen is almost twice as long as the preceding ones, and is furnished with two teeth on the lower border on either side: the anterior tooth is pointed; the posterior, blunt.

The eye-stalks are clavate, and less than one-half as long as the antennary segment.

The peduncle of the first antennae is composed of a basal segment nearly equal in length to the ocular peduncle, and two short segments. The proximal end of the basal segment is slightly enlarged for the accommodation of the auditory sac with its enclosed otolith. (Figure 1, ε.) (Note 2.) The peduncle bears a long multiarticulate flagellum, the proximal annuli of which are furnished with short setae.

The peduncle of the second antennae is composed of two segments. Of these, the first is very short, and bears a short "olfactory denticle." The second segment is much longer, but not equal to the proximal segment of the first



antennae. The flagellum is apparently about as long as the flagellum of the first pair. The second antennae bear at their base, externally, an "antennal scale," (Figure 1, *d*,) which is fringed with numerous setae, and equals the eye-stalks in length.

The mouth is bounded in front by a large labrum, (Figure 1, *e*; Figure 2, *a*,) then follow a pair of mandibles, (Figure 2, *b*,) and a bilobed metastoma. (Figure 2, *c*,) The first maxillae consist of a small setiferous inner lobe, (Figure 2, *d''*,) a larger outer lobe (Figure 2, *d'*,) also armed with setae, and a palpus. (Figure 2, *d'''*,) The second maxillae (Figure 2, *e'*,) possess a "scaphognathite," (Figure 2, *e''*,) but their structure was not made out in detail. The first maxillipeds (Figure 2, *f*,) are two-jointed, the terminal segment beset with setae on its inner border.

The second maxillipeds (Figure 1, *f*, Figure 2, *g*,) are made up of six segments, the three distal bent back upon the preceding ones. All the segments of this appendage and the four following pairs bear scattered setae.

.The four following pairs of appendages (third maxillipeds and first, second and third "deca-podal" legs,) are bent forward. The second pair is the shortest, next in length comes the first pair, next the third, the last being the longest, and furnished with a minute claw at the extremity.

There is no trace of the fourth and fifth pair of "decapodal" legs, nor of outer branches on any of the thoracic pairs.

The first pair of abdominal appendages in this (male) specimen are armed with the peculiar prehensile organ (Figure 1, *m'*.) which is commonly found in the males of this genus. It consists of a movable piece (Figure 3, *a*.) which closes upon a blunt process, (Figure 3, *b*.) tipped with minute teeth. There is but one terminal branch.

The second pair of abdominal appendages have three terminal branches, (Figure 1, *n'*, *n''*, *n'''*.) the remaining four pairs possess two terminal branches. The outer branch of the last pair (Figure 1, *r'*.) is longer than the inner branch, (about one-third longer than the telson.) and is produced at postero-lateral angle into a sharp tooth. The terminal branches of all the abdominal limbs are furnished with setae, excepting the short plate-like third branch of the second pair, (Figure 1, *n'''*.)

Length. 9 millimetres.

The single specimen obtained agrees in most respects with the description of the earliest known species by J. R. Thompson, (Note 3.) It differs noticeably, however, in the shorter eye-stalks. In this it agrees better with *Lucifer Regnaudii*, M. Edw., (Note 4.) In view of the unsatisfactory description of the known species, I have thought best not to impose a new specific name upon this

specimen until sufficient material is at hand for a critical revision of the species.

CAMBRIDGE, January 9th, 1879.

#### NOTES.

1. Report on the Dredgings in the region of St. George's Banks, in 1872. By S. J. Smith and O. Harger. Trans. Conn. Acad. Arts and Sci., III, 26, 1874.

2. The auditory apparatus of *Lucifer* was first observed by SOULEYET, (Comptes Rendus, XVII, 665, *note*, 1843; Froriep's Neue Notizen, XXVIII, 84, *note*, 1843.) Later it was described and figured by HUXLEY. (Notes and Observations made on board H. M. S. Rattlesnake during the years 1846-50. Ann. Mag. Nat. Hist., 1851, p. 305, Pl. XIV, Fig. 1.) *Cf.* also, KRÖYER, Forsøg til en monographisk Fremstilling af Kræbsdyrslægten Sergestes. Med Bemærkninger om Dekapodernes Horeredskaber. Kong. Dansk. Vidensk. Selsk. Skrifter. V, Naturvidensk og Math., Afd. IV, 293, Tav. V, Fig. 20, 1859. HEUSEN, Studien über das Gehörorgan der Decapoden. Zeits. Wiss. Zool., XIII, 383, 1863.

3. Zoölogical Researches and Illustrations, p. 58, Pl. VII, Fig. 2, 1829. Thompson's specimen was taken in the Atlantic, Lat. 11° 56' N., Long. 32°

55' W. He described it under the generic name simply. The trivial name *typus* was given later by Milne Edwards, (Hist. Nat. des Crustacés, II, 469, 1837.)

4. *Loc. cit.*, Pl. 26, Fig. 10. From the Indian Ocean.

Besides the above cited works, *cf.*, with reference to *Lucifer*, DANA, Crust. U. S. Explor. Exped., pp. 668-675, Pl. 44, Fig. 9; 45, Figs. 1-3, 1852.

SEMPER, Zeits. Wiss. Zool., XI, 106-107, 1862.

CLAUS, Ueber einige Schizopoden und niedere Malacostruken Messina's. Zeits. Wiss. Zool. XIII, 433-437, Taf. XXVIII, Fig. 21-26, 1863.

A. DOHRN, Untersuchungen über Bau und Entwicklung der Arthropoden. Zeits. Wiss. Zool., XXI, 356-359, Taf. XXVII, Fig. 1-10, 1871.

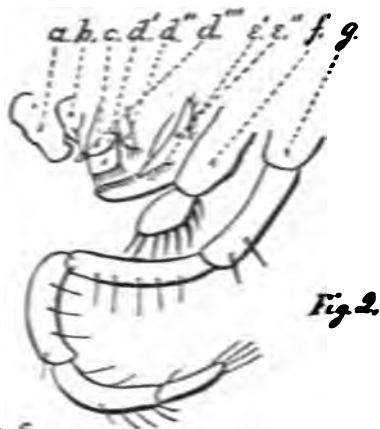
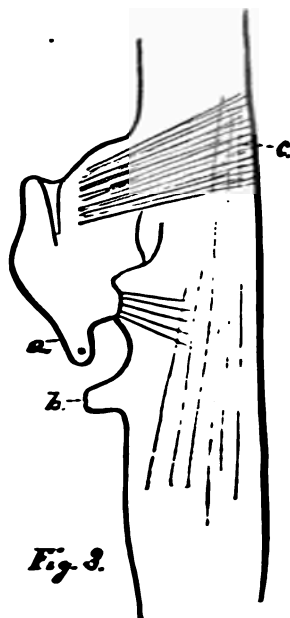
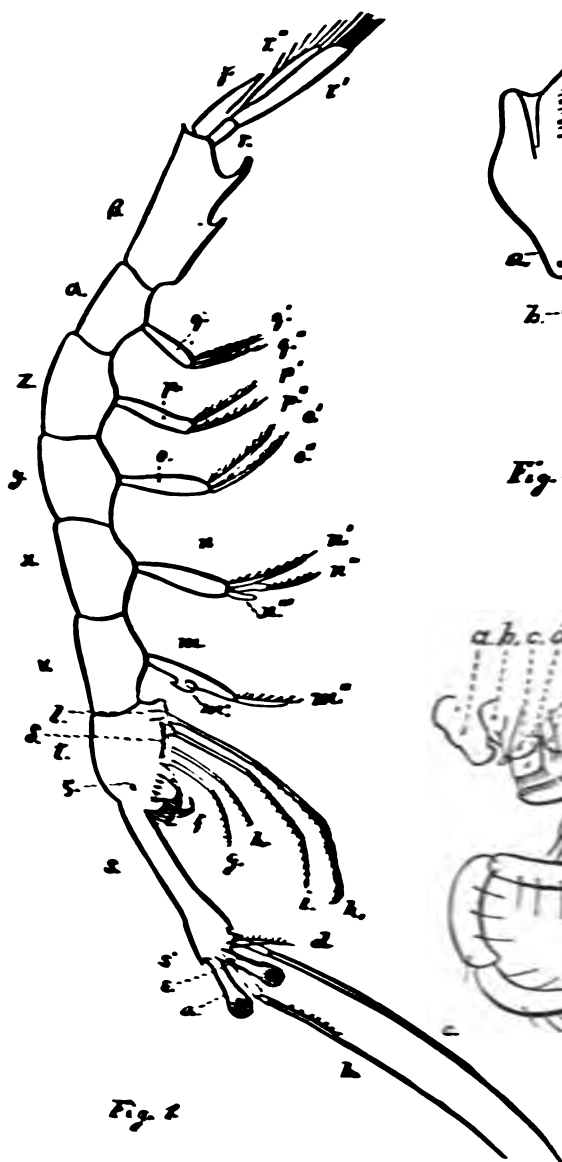
WILLEMÖES-SCHM, "Preliminary Remarks on the Development of some Pelagic Decapods." Ann. Mag. Nat. Hist., XVII, 163, 1876. (An interesting note on the development of *Lucifer*, showing that Dana's genus *Erichthina* is a young stage of *Lucifer*. See also, CLAUS, Untersuchungen zur Erforschung der genealogischen Grundlage des Crustacien-Systems, p. 40, 112, 113, 1876.)

STREETS, Contr. to the Nat. Hist. of the Hawaiian and Fanning Islands and Lower California Bull. U. S. National Museum, No. 7, p. 122, 1877.



*Lucifer typus.*

**Plate 7.**



WALTER FAXON & W. E. R. DEL.

## EXPLANATION OF FIGURES.

**FIGURE 1.**—*Lucifer typus* M. Edw.? 9 mm. in length. *a*, eye-stalk; *b*, antenna of first pair; *c*, antenna of second pair; *d*, "scale" of second antenna; *e*, labrum; *f*, second maxilliped; *g*, third maxilliped; *h*, *i*, *k*, appendages corresponding to the first, second and third pairs of legs of the higher Decapods; *l*, sac at the end of the *vas deferens*? *m*, first abdominal appendage; *m'*, copulatory organ on the first abdominal appendage; *m''*, terminal branch of the first abdominal appendage; *n*, *n'*, *n''*, *n'''*, second abdominal appendage with its three terminal branches; *o*, *p*, *q*, third, fourth and fifth abdominal appendages with their two terminal branches; *r*, *r'*, *r''*, basal segment, outer and inner branches of the sixth abdominal appendage; *s*, antennary segment; *s'*, spine on antennary segment; *t*, carapace; *v*, *x*, *y*, *z*, *a*, *β*, first to sixth abdominal segments; *γ*, telson; *δ*, nervous cord in the thorax; *ε*, auditory organ in basal segment of first antenna; *ζ*, spine on the carapace.

**FIGURE 2.**—Do. Mouth-parts of left side. *a*, labrum; *b*, mandible; *c*, metastoma; *d'*, outer lobe of first maxilla; *d''*, inner lobe of first maxilla; *d'''*, palpus of first maxilla; *e'*, second maxilla; *e''*, scaphognathite; *f*, first maxilliped; *g*, second maxilliped.

**FIGURE 3.**—Do. Prehensile male organ on the anterior border of the first pair of abdominal appendages,  $\frac{1}{2}$  inch objection. *a*, movable piece which closes upon the process *b*; *c*, muscles which move *a*.





# Preliminary Observations upon the Development of the Marine Prosobranchiate Gasteropods.

BY W. K. BROOKS.

THE segmentation of the egg among the Mollusca, and the early stages of development in the various groups, present so many variations that it is of the greatest importance that at least an outline of the process should be published for as many forms as possible.

During the summer of 1877 I made use of the opportunities which the laboratory of the United States Fish Commission at Wood's Hole afforded for studying the development of certain Marine Gasteropods, whose eggs are characterized by the presence of a large food yolk and the restriction of the segmentation to one pole of the egg. While my observations agreed in many points with those of Brobetsky, ("Studien über die embryonale Entwicklung der Gasteropoden," Arch. f. Mic. Anat., 1876,) my interpretation of the phenomena was totally opposite in certain important particulars. According to this observer, the mouth and foot are formed at that pole of the egg where the blastoderm finally closes together to surround the food yolk, and the definitive mouth is similar in position to the gastrula mouth. According to my observations, the mouth and foot appear upon that pole

where the segmentation commences, and the gastrula mouth coincides, in position with the shell gland.

During the past summer, at Fort Wool, I reviewed the subject thoroughly and satisfied myself of the correctness of my conclusions, and I will, therefore, give a sketch of the more important points, illustrated by outlines from the finished drawings which may not be published for some time. As the paper is simply an abstract, all general questions, disputed points and comparisons with the results of other observers will be omitted. The drawings which are copied are from the eggs and young of two of our common Prosobranchs, *Astyris lunata* and *Urosalpinx cinereus*. Figures 5, 6, 7 and 8 are early stages in the development of *Astyris*, and all the others are the eggs and embryos of *Urosalpinx*.

The eggs of *Urosalpinx* are contained in small transparent membranous parchment-like vases, each of which is attached by an expanded foot to some solid substance, usually the under surface of an overhanging rock, a little above low tide mark. Each female deposits great numbers of these vases, from ten or twelve up to more than a hundred, and the process of laying occupies several weeks. When the animal is not disturbed during oviposition the vases are all attached, in more or less regular rows, to the same surface, and in this way an area of three or four square inches may be covered.

In shape and size they are much like the well known egg cases of *Purpura lapillus*, but they have not their slight red tinge of color. They are flattened vertically, and their edges are marked by keel-like ridges. Owing to the length of the period of oviposition, eggs and embryos in all stages of development are to be found in the various vases of a group, and the young escape from the first-laid vases before the

female has finished laying. Unlike the vases of *Purpura*, each of which contains several hundred eggs, those of *Urosalpinx* contain only from six to twenty, ten or twelve being the usual number. All of these normally undergo development and give rise to embryos, although abnormal or retarded eggs are frequently found, and the rate of development varies greatly among the eggs in the same vase. Occasionally a partially segmented egg or a more advanced embryo becomes abortive and breaks up into separate cells, each of which remains alive for some time and often swims actively by the motion of its cilia. These "cosmellae" and the yolk of the aborted eggs are drawn into the digestive cavities of other embryos; but while this method of furnishing the young with food appears to be normal with *Purpura*, it is accidental and exceptional with *Urosalpinx*. The eggs are suspended in a tenacious transparent albuminous substance which at first fills the vase, but is used up as food by the growing embryo, which increases in size many hundred-fold before it leaves the capsule.

Before segmentation the eggs are nearly spherical, opaque, yellowish white, and are made up of a ground substance or network of transparent, very slightly granular protoplasm, the meshes of which are filled with highly refractive globules of deutoplasm or food material, which are packed into the protoplasm like the starch grains in a potato. The protoplasm stains readily with carmine or with osmic acid, and is then quite conspicuous. When an egg is torn to pieces with needles the spherules of deutoplasm fall out of the spaces, but retain their shape, while the protoplasm still exhibits the sharply defined spherical chambers which contained them.

The animals will not lay their eggs in confinement, and as the vases must be collected, taken to the laboratory and

opened before the eggs can be studied, the species is not a favorable one for studying the earliest changes, and its opacity prevents the internal changes which precede and accompany segmentation from being seen.

The first external change is the lengthening of one pole, so that the egg becomes pear-shaped. The portion thus drawn out now becomes less opaque than the remainder of the yolk, owing apparently to the absorption by the transparent protoplasm of some of the opaque food spherules, and we are now able to distinguish the formative from the nutritive pole. Two or three large spheres of segmentation now separate from the formative pole, as shown in Figure 1. As in many other mollusca, a well marked period of contraction succeeds each period of active segmentation, and the segmentation spherules are alternately sharply separated from the yolk, and then partially merged in it.

The spherules which first appear are less opaque than the food yolk, and one end of each of them, the end towards the left in Figure 1, is quite transparent.

We have then, at this early stage, one pole of the egg distinguished from the other by the presence of segmentation spherules; and one side of these spherules distinguished from the other by its transparency.

Since the subsequent history shows that the final mouth of the animal is formed at the nutritive pole of the egg, and that the opaque portions of the spherules are upon what is to become the dorsal surface, we may hereafter use the terms oral and aboral, dorsal and ventral. In Figure 1 the oral end is above, the aboral below, the dorsal surface to the right, and the ventral to the left. At the stage shown in Figure 1, a transparent area is visible on the ventral side of the oral end of the food yolk, *B*. It is probable, from Brobetsky's

observations upon *Nassa*, that this is to be regarded as the point where a spherule, similar to those shown at *D*, has become fused with the food yolk. As the cells of the ectoderm are to be derived in great part from this transparent area, it may be called the *ectodermal area* of the food yolk. It stains readily with carmine or osmic acid, as do the transparent ends *D''* of the spherules *D*. A group of small transparent segmentation spherules now makes its appearance at the oral end of the egg and ventral to the large spherules, and soon forms a distinct layer of ciliated cells: *the ectoderm*. This layer seems to be derived in part from the transparent ventral surfaces of the primary spherules or macromeres, but mainly from the transparent ectodermal area of the food yolk.

The macromeres *D* now divide and give rise to a number of opaque spherules, much larger than the ectoderm cells, and so arranged as to form a rim around the sides and dorsal edge of the ectoderm. As these opaque large spherules give rise to the wall of the digestive cavity, we shall call them the endoderm hereafter. Figure 2 is a ventral view, Figure 3 a dorsal view, and Figure 4 a dorso-ventral optical section of the oral portion of an egg at this stage, much more highly magnified than Figure 1. In Figure 2 the ectoderm is shown at *F* as a layer of small ciliated cells, bounded dorsally and at the sides by the endoderm spherules *D*, and ventrally by the food yolk *B*. The ventral margin *F'* of this layer now extends downwards onto the ventral surface of the food yolk, by the addition of new cells to the ventral margin *F'*. These new cells appear to be derived from the ectodermal area 1. The endoderm spherules *D* are seen at the sides of the layer of ectoderm, separating it from the food yolk, and also projecting above its dorsal edge. In

the dorsal view, Figure 3, a small portion of the ectoderm *F* is seen above these spherules *D*. In the optical section, Figure 4, the ectoderm forms a single layer, *F*, of nucleated ciliated cells, which arch over a segmentation cavity *F''*. The letter *F'* indicates the ventral growing edge of the ectoderm, and 1 the ectodermal area. A comparison of Figures 2, 3 and 4 shows that the segmentation cavity is bounded below by the surface of the food yolk *B*; dorsally and at the sides by the macromeres *D* of the endoderm, and ventrally and orally by the ectoderm *F*. The latter now grows down onto the food yolk on all sides, and covers up the endoderm spherules and at the same time pushes them down towards the aboral pole. Figure 9 is the oral end of an egg at a somewhat later stage, in which the ectoderm forms a cap upon the oral end of the food yolk *B* and nearly covers the endoderm *D*. At this stage the embryo begins to rotate slowly by the action of the cilia of the cap of ectoderm. Figure 5 is a dorsal view of the entire embryo of *Astyrus lunata* at a somewhat later stage. The food yolk *B* is now nearly covered by the cap of ectoderm *F*, which also entirely covers the endoderm *D*. The foot is now present as a fold of ectoderm upon the oral ventral edge of the embryo, and its corners are seen at *G*, projecting beyond the general outline of the oral surface.

The endoderm spherules *D* have not yet undergone very much change; they are covered by the ectoderm and are pushed down from their original position onto the sides of the food yolk, around the sides and dorsal surface of which they form an incomplete ring, open on the ventral surface, as seen in the side view, Figure 6. On the sides this belt is only one cell wide, but upon the dorsal median line it has begun to grow upwards towards the oral end of the embryo.

The embryo shown in Figure 6 is also an *Astyris*, a little younger than that shown in Figure 5, and seen from the left side, or in the same position as Figures 1 and 4. Both figures are from embryos which had been placed for half an hour in one-fifth per cent. solution of perosmic acid, and had then been stained with carmine. The endoderm is so obscured by its covering of ectoderm that it cannot be satisfactorily studied in a living specimen, but it is quite distinct in stained specimens.

In this figure the projecting foot *G* is quite prominent, and as it is the first organ to appear and is present in both *Astyris* and *Urosalpinx* before the closure of the blastoderm around the food yolk is completed, as well as at all later stages, it is of the greatest importance in determining the relation of organs of later formation to the poles of the segmenting egg.

At *F'* on the ventral surface, under the foot, is the opening in the belt of endoderm, and over it the growing edge of the ectoderm. Figure 7 is a median vertical optical section of Figure 5, and Figure 8 a similar section of Figure 6. As the letters of reference are the same as in the previous figures, they will at once be understood from the previous description.

As development progresses, the sides and ventral margin of the ectoderm continue to extend down onto the food yolk, and at last surround it, meeting at a point which corresponds in position to the point *C* of Figures 5 and 6, which in its relation to the endoderm is the same as the point *C* of Figures 3 and 4. Meanwhile the oral half of the food yolk becomes absorbed, and the layer of endoderm grows upwards upon the sides and dorsal surface of the embryo and thus builds up a wall or parapet around three sides of the now

which is the first stage of the gastrula and this process goes on until the whole surface of the walls meet at the oral end of the embryo. In this way the cavity which is left after the absorption of the food yolk becomes enclosed over by a roof of ectoderm continuous with the layer of ectoderm, but separated from it by the segmentation cavity.

The primitive digestive cavity which is thus formed is open along the ventral median line and it is bounded below by the oral surface of the food yolk and dorsally and laterally by the ectoderm. It opens externally by a "gastrula mouth," the position and mode of formation of which will be understood by a comparison of Figures 10 and 11.

Figure 11 is a median sagittal section of a Urosalpinx embryo, after the ectoderm *E* has surrounded the food yolk *B* and the oral end of the latter has been absorbed. The oral end is uppermost, and the ventral surface to the left, and it is therefore in the same position as Figures 1, 4, 6 and 8. The layer of ectoderm has passed around the aboral surface of the food yolk and up its dorsal surface as far as the point *C* near the centre of the dorsal surface. A comparison with the previous figures shows that the point *C*, which in Figure 11 is the only portion of the body which is not covered with ectoderm, is the same as the point in Figure 1 where the dorsal surfaces of the segmentation spheres join the food yolk. A view of the dorsal surface of the same embryo, Figure 10, shows that the area *C* is circular and that its margin is formed by a ring of ectoderm cells, and deeper focusing shows that the oral margin and sides of this ring are in contact with the endoderm spherules *D* and its aboral margin in contact with the upper edge of the food yolk *B*. It thus forms a circular aperture, the gastrula mouth, through which the digestive tract opens externally; it is functionally



a mouth, and food passes through it into the digestive cavity before the formation of the definitive mouth. At this stage, Figure 11, the ectoderm of the oral end of the body has become differentiated into the foot *G*, the head vesicle *K* and the invagination *I*, which is to become the definitive mouth.

This invagination does not at first reach the surface of the endoderm, and its inner end is closed. Since it makes its appearance above the foot *G*, we are able to say that it is formed at the point which is indicated by the letter *A* in Figure 6, or at one end of the long axis of the embryo. Since the food yolk is not entirely covered by the blastoderm at this stage, (Figure 6,) it is plain that the mouth invagination is formed at that pole of the egg where segmentation commences, and which we have called the oral pole.

At the stage shown in Figures 10 and 11, the endoderm *E'* has grown upwards dorsally and at the sides, and the digestive cavity *E''* is nearly shut in, although there is still an uninclosed belt or zone along the ventral median line, represented by dotted lines in Figure 11. The greater part of the wall of the digestive cavity is so filled with small vacuoles that the outlines of the constituent cells could not be traced; but along the dorsal and lateral edges, where it meets the food yolk, the large endoderm spherules *D* can still be seen. In the dorsal view of the same embryo, Figure 10, the primitive urinary organs *H* are seen at the sides of the body, covered with a single layer of small nucleated polygonal cells and filled with a loose mass of nearly spherical cells, the origin of which was not traced.

At a later stage of development, the endoderm meets along the ventral median line, and the oral end of the embryo is then composed of two nearly concentric layers of cells, the

ectoderm and endoderm, separated by a body cavity, which appears to be identical with the cavity of segmentation. For some time the floor of the digestive cavity is the surface of the food yolk *B*, but in time the endoderm grows inwards, as shown by the dotted lines at *E'* in Figures 10, 11 and 12, and separates the food yolk from the cavity. Before this floor is completed, the albumen of the egg capsule is drawn into the digestive cavity, through the gastrula mouth, apparently by ciliary action. The cavity becomes greatly distended and the long axis of the embryo lengthened, as shown in Figure 12.

The ectoderm is stretched by this inflation, and the foot *G*, Figure 12, and the mouth invagination *I* are thus rendered less conspicuous than at an earlier stage. The endoderm, on the contrary, absorbs nutriment from the food, thickens, and becomes filled with great numbers of oil-like vacuoles, and is more conspicuous than at an earlier stage.

Along the dorsal portion these vacuoles unite and form one large one for each cell, and the outlines of the separate cells become visible. The velum *L* makes its appearance as a band of large nucleated cells, with long cilia, and running across the anterior end of the body, dorsal to the mouth invagination. At *H* one of the primitive kidneys is shown, and it will be seen that it is much nearer the anterior end of the body than in Figure 10. At this stage the floor *E'* of the digestive tract is not quite completed, and near the dorsal surface a small portion of the food yolk is still exposed. The margins of the gastrula mouth are now greatly thickened, and before the floor of the digestive tract is quite completed, the opening is obliterated and its margins become the shell gland, Figure 12, *C*, upon which a small circular transparent shell, *M*, soon appears. The closure of the gastrula mouth and formation

of the shell take place before the invagination *I* of the true mouth unites with the endoderm.

Figure 13 is a somewhat older embryo, less highly magnified than Figure 12, but in nearly the same position. The marked change in the general form and outline of the body is the result of a ventral flexure of the long axis. The dorsal surface is thus lengthened, the ventral shortened, and the oral and aboral poles brought nearer each other.

The ectoderm is now continuous over the spot *C*, where the gastrula mouth was situated at an earlier stage, and the shell, which has now increased greatly in size, has moved from its primitive position over the point *C* and is now a symmetrical circular cap, *M*, upon the convex dorsal surface of the bend in the body of the embryo. Around its edge is a thickened ridge, *R'*, the rudimentary mantle.

The food yolk *B* is of about the same size as in Figures 10, 11 and 12, but it is now entirely shut off from the digestive cavity *E''* and lies between the integument *F* and the endoderm *E'*.

The mouth invagination now communicates by a short ciliated œsophagus, *I*, with the digestive tract, and the embryo draws into its stomach the yolk of abortive eggs and fragments of other embryos, as well as the transparent albumen. As the solid particles are drawn through the œsophagus, they are pressed and twisted into long strings, *N*, which are frequently to be found in the digestive cavity. The foot *G* is now quite conspicuous, and the velum *L* begins to bend towards the dorsal surface. The embryo now grows very rapidly, and at the stage shown in Figure 14, is three or four times as large as that of Figure 13, and is represented in substantially the same position. Up to this time the embryo has been bilaterally symmetrical, but the symmetry

is now departed from by the twisting of the aboral pole towards the right side. In a side view, as in Figure 14, the outline is roughly triangular; one angle being formed by the head vesicle *K*, another by the food yolk *B*, and the third by the bend in the dorsal surface which is covered by the shell *M*; the dorsal surface forms two of the sides of the triangle, and the third, more broken side is formed by the outlines of both the ventral surface and the oral end of the embryo. Owing to the twist which is mentioned above, the front view of the embryo is no longer symmetrical. At the earlier stage shown in Figure 13, a view from in front, or along the line *EB*, would be perfectly symmetrical, and the food yolk *B* would be hidden behind the oral end of the body. In a similar view of Figure 14, that is a view along the line *KB*, the whole of the food yolk *B* would be seen to the right of the head vesicle *K* and the velum *L*, as is shown in the somewhat older stage, Figure 15. In Figure 14 the shell *M* is still nearly symmetrical, and still rests like a cap upon the rounded angle formed by the flexure of the dorsal surface. The organs at the oral end of the body are now quite highly developed. The foot *G* is a large projection which contains a cavity, which is traversed in various directions by a network of contractile cells, with the central nucleated body and radiating processes so characteristic of the interstitial connective tissue corpuscles of the mollusca. Among them a few free white blood corpuscles may occasionally be seen; and as the foot changes its shape, through the contraction of the radiating processes, the blood corpuscles are driven from one point to another. The head vesicle *K* is similar to the foot in structure, but its cavity is larger, its contractions more regular, and its functions as an embryonic heart much more efficacious. No trace of a dis-

tingent layer of mesoderm, such as is readily recognized in this region of a fresh water pulmonate at this stage, could be detected. The velum *L* is now well developed, and its free ends nearly meet upon the dorsal surface of the neck. In a view from in front the halves of the velum are seen to project from the sides of the body, as in those Gasteropods where the veliger embryo leads an active life. The oesophagus is now a long cylindrical ciliated tube, slightly bent upon itself, so that the convex side of the bend is dorsal. The digestive tract is still a large unspecialized chamber, which fills nearly the whole embryo. During growth the endoderm cells continue to grow more conspicuous, and they also increase greatly in size, and are represented in Figure 14 about as large as in Figure 12, although the latter embryo is much smaller and more highly magnified.

The endoderm and ectoderm are in contact over the greater portion of the surface, except posteriorly, where they are separated by the food yolk *B*, and at the oral end of the body. At the point *O* a ciliated depression indicates the point where the anus is to be formed, and a thickening of the ectoderm, not shown in any of the figures, runs inwards to form the rectum. This is at first a solid cord, and it becomes hollow and communicates with the digestive tract at a later stage. I was not able to determine how much of the intestine is formed from this plug of ectoderm.

By a comparison with the previous figures, the point *O* will be seen to be separated by the width of the food yolk from the point where the gastrula mouth was situated.

The subsequent changes of the now rapidly developing embryo are too complicated to be described without carefully finished drawings, but outlines are given of four of the most characteristic of my figures of the later stages.

Figure 15 is an embryo older and much larger and less highly magnified than Figure 14, and viewed from in front or along the line *KB*; in Figure 14 and Figure 16 a still older and larger embryo is shown from behind. The two folds of the velum, *LL*, now project considerably from the sides of the neck, and the relation of this organ to the head vesicle and foot will be best understood by a comparison of Figures 14, 15 and 16. In Figure 14 the head vesicle *K* is dorsal to the mouth, and in Figure 15 it is a nearly spherical chamber, *K*, in front of the velum *L*. In Figure 16 part of its outline, *K*, is seen in front of the velum. In Figure 15 the œsophagus *I'* and the buccal cavity *I* are seen through the head vesicle, and beyond them is the foot *G* and the outline of the neck. The primitive kidneys have now passed forward and project from the sides of the neck at *H*, in Figures 14, 15 and 16. In Figure 16 the foot *G* is a large creeping surface, anterior to which is the mouth opening *I*; at the point where the head vesicle joins the velum, the tentacles *V* have now made their appearance. In both figures the food yolk *D* is seen a little above the velum and upon the right side of the embryo. It is a little difficult to use the terms right and left in the description of an irregularly twisted form, but it will be seen that if either of these figures were rotated until the creeping surface of the foot were below, and the head vesicle anterior, the food yolk would be on the right side. In Figure 15 the shell is still nearly symmetrical, although it has increased greatly in size and now covers all of the body, except the portions which formed the two ends of the embryo in Figure 10. The endoderm cells have increased greatly in size, and are of the same apparent size as in the more magnified figures of earlier stages. Around the margin of the shell there is, as in earlier stages, a thickened

rim, the mouth, and anterior to this, on the right side of the dorsal surface of the neck, the integument becomes folded at *S* to form the mantle chamber. The edges of this chamber are slightly scalloped, and the projecting points are the beginnings of the gill filaments. Within this cavity a large rhythmically pulsating organ, *T*, the embryonic heart, now makes its appearance. The shell now becomes asymmetrical, the right side of the dorsal margin, that is the margin nearest the mantle cavity *S*, growing most rapidly; and the shell soon assumes a spiral form, as shown in Figure 16. A comparison of this with the preceding figure shows that the lip of the shell of the adult is the right margin and the columella the left margin of the embryonic cup-shaped shell. In the stage shown in Figure 16, the margin of the columella is bent outwards at *U*, thus forming a fold, which lies upon the posterior face of the body, or neck, just above the foot. This latter organ is at this stage placed at right angles to the long axis of the aperture of the shell, but it soon rotates so as to be parallel to this axis, as shown in Figure 18; at the same time it increases in size and becomes about as long as the shell. By this rotation and growth the upper surface of the foot is brought into contact with the columellar flap *U*, which grows and becomes the operculum. For some time this is united to the shell by an elastic hinge or line of flexure. When the foot is withdrawn into the shell, the operculum bends down with it into the aperture, bending along the line where it is reflected outwards from the columella, but it soon becomes separated from the shell, and the growth of the foot carries it away from the columella, as shown in Figure 18. The velum begins to become rudimentary at about the stage shown in Figure 16, and at the stage shown in Figure 17 it is quite small, and in Figure 18, the

stage in which the animal escapes from the capsule, it has disappeared.

The large food yolk and the supply of food contained in the vase are sufficient to carry the young animal up to the true gasteropod form, and the free-swimming veliger stage of development is omitted, although the tendency to develop a velum is still retained.

The changes which we have described may now be briefly recapitulated, as follows:

Segmentation takes place at one pole, the oral pole, of the large yolk and results in the formation of a blastoderm.

Two kinds of segmentation spherules are distinguishable at a very early stage: large opaque spherules, which ultimately give rise to the wall of the greater part of the digestive tract, and much smaller transparent spherules, which soon become ciliated and form a layer ventral to the endoderm and arching over the segmentation cavity. The endoderm spherules become arranged in a band around the sides and dorsal margin of the food yolk, and the layer of ectoderm extends over them and also down onto the ventral surface of the food yolk. The endoderm is also pushed down onto the sides of the food yolk with the growth of the ectoderm.

The foot now makes its appearance at the oral ventral end of the embryo.

The ectoderm surrounds the food yolk, and forms the margins of the gastrula mouth, upon the dorsal surface.

While the digestive cavity still opens externally by the gastrula mouth, the true mouth invagination makes its appearance at that end of the embryo where the segmentation began. The digestive cavity is formed by the absorption of the oral half of the food yolk, and the walls of the cavity are derived from the macromeres. For some time the aboral floor



of the digestive tract is formed by the surface of the food yolk, but the edges of the endodermal wall soon become reflected inwards and at last form a continuous floor which separates the food yolk from the digestive cavity. Before this floor is completed, or the true mouth communicates with the cavity, the margins of the gastrula mouth become thickened to form the shell gland, and the opening disappears. Soon afterwards the true mouth is formed, and later the anus and intestine, and a considerable portion of the latter is derived from the ectoderm.

At first the body is long, cylindrical and bilaterally symmetrical, and the mouth is at one end and the shell upon the dorsal surface, but it soon bends upon itself so as to shorten the ventral surface and bring the extremities nearer each other, and a second twist carries the aboral end of the body onto the right side, and the most posterior portion of the body is now the middle of the dorsal surface.

A velum is now developed, but soon lost, and the animal leaves the egg case as a true Gasteropod.

The developmental history here traced is of especial interest in its relation to the gastrula theory. The typical gastrula stage, resulting from total regular segmentation, as in the Echinoderms and Paludina or Cyclas, is an elongated double walled vase, with an opening at one end, and an axially elongated cavity.

The outer wall of transparent ectoderm is not differentiated into organs, and it unites with the endoderm, which is also undifferentiated, around the margins of the aperture.

It is clear that no stage in the present development is a typical gastrula stage, nor is there any stage which can be regarded as a specialized gastrula stage complicated by the formation of other organs; but a little study will show that

the embryo presents at different periods all the phases in the formation of a gastrula, although there is no time when all the characteristics of a gastrula exist together. The gastrula *stage* has disappeared, but the gastrula *form* persists, and may be recognized by neglecting all those complications of structure which do not take part in its formation. Suppose, for instance, that the differentiation of the ectoderm of the oral end of the body, which in Figure 10 indicate the positions of the foot, mouth, velum and head vesicles, did not make their appearance until a later period, we should then have at this stage an undifferentiated layer of ectoderm, entirely surrounding the embryo, except at the point *C*. At the same time, imagine the development of the wall of the digestive tract accelerated, and the cavity entirely separated from the food yolk, before the specialization of the gastrula mouth as a shell gland.

We should then have a central digestive cavity, surrounded by two layers of cells, widely separated on one side by the food yolk.

If this were away, the embryo would then be a typical radically symmetrical gastrula. If the food yolk were wanting, the development of the wall of the digestive tract accelerated, the development of the foot, mouth invagination, velum and head vesicle retarded, we should have a true gastrula; or, conversely, the acceleration of the development of the latter organs, and the retardation of that of the digestive cavity, and the presence of a food yolk, might so modify a typical gastrula, as to give us the form of development which we here find.

## DESCRIPTION OF THE PLATE.

All the figures are from the embryos of *Urosalpinx cinereus*, excepting Figs. 6, 7, 8 and 9, which represent an early stage in the development of *Astyris lunata*.

The letters of reference are the same for all the figures, and are as follows :

- A. The *Germinative* or *Oral Pole* of the embryo.
- B. The unsegmented *Food Yolk* at the *Nutritive Pole* of the embryo.
- C. The point upon the dorsal surface of the embryo, where the *gastrula mouth* is situated during the stage of development shown in Fig. 11. At the stage shown in Fig. 12, the *shell-gland* occupies the area where the *gastrula mouth* was situated a little earlier.
- D. The large opaque segmentation-spheres, which form the *Endoderm*.
- D'. In Fig. 1, the more opaque dorsal endodermal portion.
- D''. In Fig. 1, the transparent ventral ectodermal portion.
- E. The endodermal wall of the digestive cavity.
- E'. The portion of this wall which is reflected onto the oral surface of the food yolk.
- E''. The cavity of the embryonic stomach.
- F. The *Ectoderm*.
- F'. That margin of the layer of ectoderm which grows down onto and around the food yolk.
- F''. The *Segmentative Cavity*.
- G. The *Foot*.
- H. The *Primitive Renal Organ*.
- I. The *Definitive Mouth*.
- K. The *Head Vesicle*.
- L. The *Velum*.
- M. The *Shell*.
- N. A mass of food which has been drawn through the mouth into the digestive cavity.
- O. The *Anus*.
- R'. The *Mouth*.

- S. The mantle cavity.
- T. The embryonic heart.
- U. The operculum.
- V. The tentacles.

FIGURE 1.—An egg, at a very early stage of segmentation, seen from the left side and magnified 100 diameters.

The egg is considerably lengthened along the axis which passes through the area of segmentation. Two segmentation spherules are sharply defined and project from the dorsal side of the oral end of the food yolk. Each of these is divided into a dorsal opaque granular portion, which is to give rise to the spherules of the endoderm, and a more transparent ventral portion from which the ectoderm is to be, in part, derived. A third segmentation spherule has become fused with the food yolk, ventrally to the two projecting spherules, and is destined to give rise to the greater part of the ectoderm.

FIGURE 2.—The germinative pole of an egg, at a later stage of development, viewed from the ventral side and magnified 250 diameters.

The food yolk, only a small part of which is shown in the figure, has regained its nearly spherical shape, and on its surface, just below the ventral edge of the layer of ectoderm, is seen the sharply defined transparent area which gives rise to new ectoderm spherules. Behind the layer of ectoderm *F*, the upper edges of the larger endoderm spherules are seen, and the smaller endoderm spherules *D, D*, extend around onto the sides of the area of ectoderm.

FIGURE 3.—The same egg, viewed from the dorsal surface.

The layer of ciliated ectoderm is now seen at *F*, projecting beyond the endoderm spherules *D, D*.

**FIGURE 4**—Optical section of the same egg. Its dorsal side is towards the right.

By the addition of new spherules, derived from the food yolk, at *F'*, the ventral edge of the layer of ectoderm gradually extends down onto the food yolk, while its dorsal margin extends towards *O*, over the endoderm *D*, by the addition of spherules derived from the latter.

**FIGURE 9**.—This figure should come next in order, but, by an oversight, it has been incorrectly numbered. It is a ventral view of the oral pole of an egg at a later stage than Figure 2.

The layer of ectoderm *F* has spread over the endoderm spherules *D*, and begins to push them down onto the sides of the food yolk.

**FIGURE 5**.—The embryo of *Astyris lunata*, viewed from the dorsal surface, at a later stage than Figure 9.

The layer of ectoderm has spread over the food yolk *B*, so as to nearly cover it. It has also covered the endoderm spherules *D* and pushed them down onto the sides of the food yolk.

**FIGURE 6**.—The same embryo, seen from the left side.

**FIGURE 7**.—Optical section of Figure 5.

**FIGURE 8**.—Optical section of Figure 6.

**FIGURE 10**.—Dorsal view of a still older embryo of *Urosalpinx*.

The ectoderm now entirely surrounds the food yolk, except at the point *C*, the gastrula mouth. The primitive kidneys *H* project from the sides of the body.

**FIGURE 11**.—Longitudinal section of the same embryo, in the same position as Figures 4, 6 and 8.

The head vesicle *K*, the mouth invagination *I*, and the foot *G*, have now appeared at the end opposite the food yolk *B*.

**FIGURE 12**.—An older embryo in the same position, showing, in addition to other features, the velum *L*, the shell gland *C*, and the shell *M*.

**FIGURE 13.**—Similar view of an older embryo, magnified only 150 diameters.

The gastrula mouth has disappeared, the true mouth *I* has been formed, and the digestive cavity *E''* contains strings of food *N* which have been drawn into it through the short œsophagus.

**FIGURE 14.**—A nearly similar view of a still older embryo, magnified 100 diameters.

**FIGURE 15.**—The anterior aspect of an older embryo.

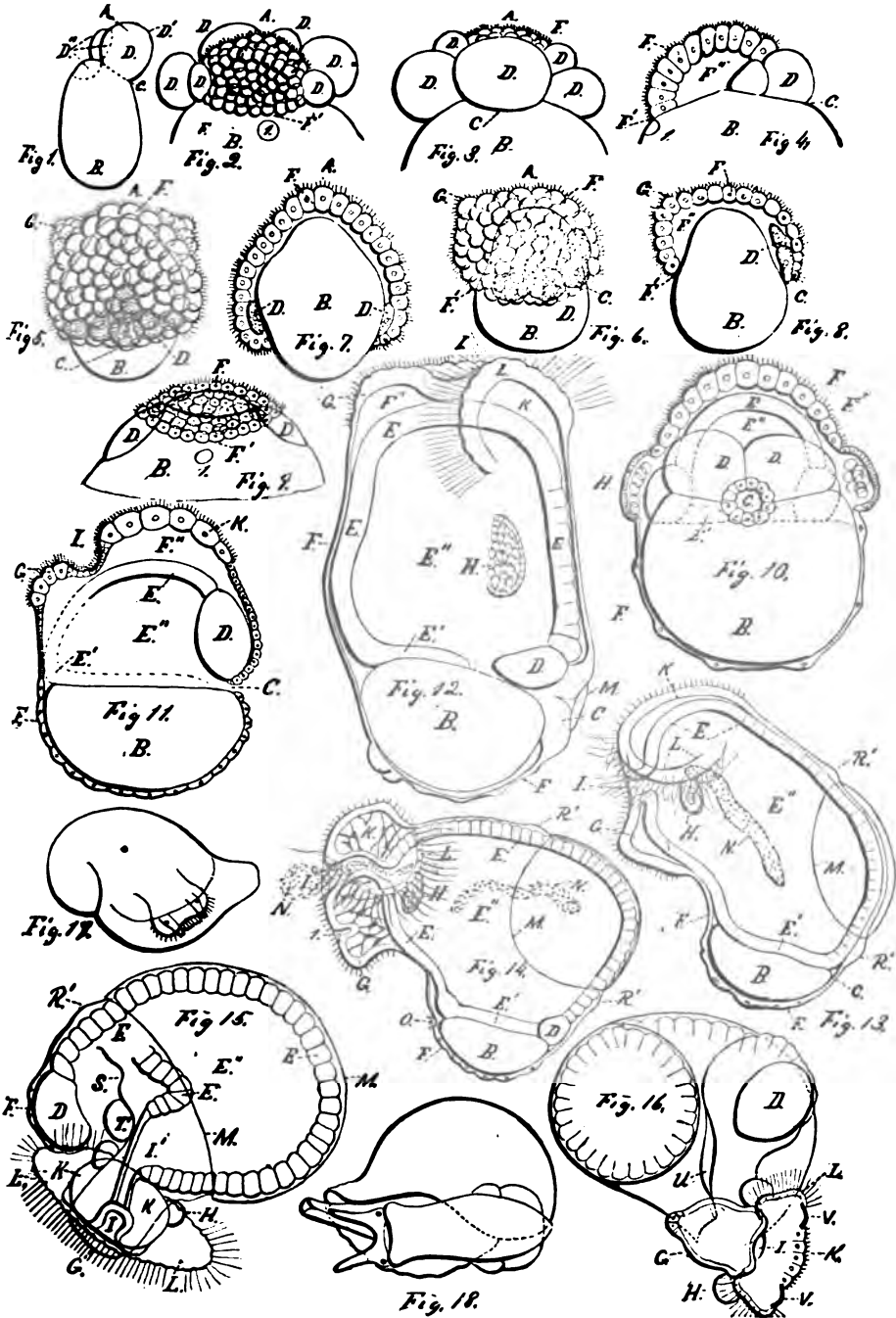
**FIGURE 16.**—Still older embryo, viewed from below, magnified 75 diameters.

**FIGURE 17.**—The young gasteropod retracted within its shell.

**FIGURE 18.**—An older one, expanded, and seen from below. This represents the stage at which the young usually leave the capsule.

**Development of Gasteropods.**

**Plate 8.**







## The Larval Stages of *Squilla Empusa*. Say.

BY W. K. BROOKS.

*SQUILLA empusa*, the only described Stomatopod found upon our coast, was described under the above name by Say, (Journal Acad. Nat. Sci., Philadelphia, vol. I, p. 224, 1818,) and again described, under the same name, and figured by Dekay, (Nat. Hist. of New York, 1844, p. 32, Pl. 13, Fig. 54,) and again described, under the same name, by Gibbs, (Proc. Amer. Assn., 3d meeting, p. 199.) According to Milne Edwards, (Hist. Nat. des Crust.,) the points of difference from *Squilla mantis* are very slight, but they are sufficient, according to the above writers, to make it a distinct species. It is met with occasionally on sandy bottoms from Cape Cod to Florida, and its free-swimming larvae are occasionally captured at the surface at Wood's Hole, Newport, and other points along the New England coast, but it is not usually found in abundance.

At Fort Wool the young were frequently met with by hundreds, and an evening's collecting always yielded several specimens. The adults

appear to be quite numerous in the Chesapeake Bay, and I am told by Mr. Jesse Price, that it is often captured in abundance upon the sand beaches near Old Point Comfort, in the fall, and he tells me that it is known to the fishermen as the sand shrimp and is eaten. The average length is from two to four inches, but much larger individuals are frequently found.

In his report upon the "Invertebrate Animals of Vineyard Sound," (U. S. Fish Commission Rep. 1871 and 1872, p. 369,) Verrill gives the following account of the structure and habits of the adult:

"The *Squilla empusa* is a very interesting creature, whose habits are still imperfectly known. It is often thrown on the beaches by the waves, and probably it usually burrows in the mud, below low-tide mark, but in certain localities it has been found burrowing at or near low-water mark of spring tides, forming large, irregular holes. . . . Large specimens are eight or ten inches long, and about two broad."

(Plates 4 and 5 of the present paper give a dorsal and a ventral view of the animal and are of about the same size as a moderately large specimen.)

"The body is not so stout built as that of the lobster, and the carapax or shell is much smaller and softer, while the abdomen is much larger and longer in proportion. The legs and all the other organs are quite unlike those of the lobster, and the last joint of the great claw, instead of forming a pair of pincers with the next, is armed with a row of six sharp-curved spines, which shut into corresponding sockets, arranged in a groove in the next joint, which also bears smaller spines. By means of this singular organ they can hold their prey securely, and give a severe wound to the human hand if handled incautiously. It also uses the stout caudal appendages, which are armed with spines, very effectively. The colors of this species are quite vivid, considering its mud-dwelling habits. The body is usually pale green, or yellowish green, each segment bordered

posteriorly with dark green and edged with bright yellow; the tail is tinged with rose, and mottled with yellow, and blackish; the caudal lamellae have the base and spines white, the last joint yellow, margined with black; the inner ones are black pale at base; the eyes are bright emerald green; the inner antennae are dark, with a yellow band at the base of each joint; and the flagellum is annulated with black and white."

The group Stomatopoda, of which *Squilla* is a representative, is quite small and sharply defined, and while its representatives are very much like the Decapods in most respects, they differ not only from them, but from all other Crustacea, in certain characteristics, such as the possession of a long tubular insect-like heart, and in having the eyes and antennae carried upon distinct, movable body segments.

Their adult structure is of the greatest morphological interest, and a knowledge of their development is of especial importance, as we are here presented with what may be regarded as the simplest expression of the highly complicated metamorphosis of the higher Crustacea. Whether the view advocated by Claus, (*Genealogischen Grundlage des Crustaceen-Systems*), as to the origin and significance of the zoea stage, be or be not correct, it is nevertheless true that the Squilloid zoea bears a more intimate relation to the adult than is the case with other stalk-eyed Crustacea, and the development of *Squilla* is therefore an excellent basis for comparative work in this department.

The station at Fort Wool presents unrivalled facilities for the study of Crustacean development, and I hope that these opportunities will in future seasons be utilized for the advancement of our comparative knowledge of the subject. Although my observations upon *Squilla* were not as exhaustive as another season's work might have rendered them, it seemed best to have them published as a basis for such future comparative work, and I have therefore selected them for publication from among the notes on Crustacea made during the summer.

While the literature upon the development of this highly interesting group is very scanty, it is sufficient to show that the adults of the various genera and species are much more alike than their larvae. This reversal of the general morphological law that allied animals become more different from each other as they pass from the egg to the adult form, is not unusual among the Crustacea. The larvae of a number of Stomatopods have been described as adults under the generic names, *Alima*, *Erichthus*, *Squillerichthus* and *Erichthoidina*, but the first description of a Squilloid larva, as such, is by Fritz Müller, (*Bruchstück zur Entwicklungsgeschichte der Maulfüßer*, Arch. f. Naturgeschichte, XXVIII, 1862, p. 353, and XXIX, 1863, p. 1.)

He figures two larvae, XXIX, Taf. I, Fig. 1, and XXVIII, Taf. XIII, Fig. 1, which are very

different from each other in form, and in the number of segments and appendages. He thinks it probable that the one figured in the first paper is derived from that figured in the second, and that they are successive stages in the development of one species.

The smallest larva, described in the second paper, is an "Erichthoidina." The transparent body is divided into three regions of nearly equal length: the anterior region is unsegmented; carries a pair of large sessil compound eyes; a median ocellus; two pairs of short jointed unbranched antennae, and the mouth parts. The second region of the body is made up of five segments, each of which carries a pair of long two-branched swimming feet. The first and second regions are covered by a large carapace, with a median frontal, and two posterior lateral spines. The third region is made up of three segments without appendages, and a large terminal plate or telson.

The second larva, an "Alima," is similar in all essential points to that shown in Plate 9, Figure 3, of the present paper. As he was unable to trace the metamorphoses of the first form into the second, or of either into the adult, his view that they belong to the same series is only a conjecture, and, according to Claus, an erroneous one. This observer, (*Die Metamorphose der Squilliden*,

von Prof Dr. C. Claus, Nachrichten von der königl. Gesellsch. zu Göttingen, 1871, 6, p. 169,) was led by the examination of a large collection of preserved material, to the view that there are two quite distinct forms of development among the Stomatopods.

He found a number of larvae in the same stage of development as that figured in Müller's second paper. These larvae were alike in all essential features, but could be separated, by differences of outline, into four sets, each of which probably represented a distinct species. With each of these forms a larger and more advanced larva, with the same specific characteristics, but in the "Erichthus" stage, was found associated, and Claus accordingly infers that the "Erichthoidina" develops into an Erichthus. Associated with one of these four forms he found a still more advanced larva, with the same specific characteristics, but in the form of the old genus *Squillerichthus*, and this again was joined onto a young *Squilloid*, with the characteristics of the adult genus *Gonodactylus*.

Claus accordingly concludes that Müller's *Erichthoidina* is the youngest post-ovian stage in the development of the genus *Gonodactylus*, and that the adult form is reached in this genus by a very complex metamorphosis, during which the animal passes through stages which had previously been regarded as adults, and had

been described under the generic names *Erichthus* and *Squillerichthus*.

Claus also found the "Alima" larva, which is figured in Müller's first paper, associated with a similar, but more advanced Squilloid larva, the larger and older specimens of which exhibited the characteristics of the adult of the genus *Squilla*, and he infers that "Alima" is the young of *Squilla*, which accordingly passes through a metamorphosis, which is very much more direct than that of *Gonodactylus*.

From some unpublished observations by Faxon, which I have had the opportunity to examine, I am led to believe that these two forms of development are not the only ones which will be found to occur among the Stomatopods, and that this very complicated department of embryology demands careful observation of the actual metamorphosis of the larva, in as many species as possible; a work which can be carried on no where but at a sea-side laboratory. My observations, however, tend to substantiate the second part of Claus' conclusion; that the Alima form is the young of the genus *Squilla*, and that it is transformed into the adult by a pretty direct metamorphosis.

Claus' paper, above referred to, is not illustrated; but he published a year after a second paper, to which I have not been able to refer,

(Die Metamorphose der Squilliden. Abhandlungen der königl. Gesellschaft der Wissenschaften zu Göttingen, 1871,) which I believe is illustrated. In his "Untersuchungen zur Erforschung der Genealogischen Grundlage des Crustaceen-Systems, 1876," he gives a short abstract of this paper, pp. 3 and 4, illustrated by three figures, p. 4, Figures 4, 5 and 6, of the second or simple form of development. The larvae figured are specifically distinct from those described in the present paper, but they are substantially the same; and the more complete series which I have have had the opportunity to study, as well as the fact that I have found an earlier stage than the youngest one described by Claus, render it pretty certain that the Erichthoidina stage is lacking in the genus *Squilla*, and that the individuals of the genus leave the egg as an Alima, which is developed into the adult form without any great metamorphosis. The only other published account of Squilloid larvae, with which I am acquainted, is by S. J. Smith. In his paper, on "The Metamorphosis of the Lobster, and other Crustacea," U. S. Fish Commission Report for 1871 and 1872, p. 536, he gives a description and a figure of a single stage in the development of *Squilla empusa*; a stage which is a little earlier than that shown in Plate 11, of the present paper, and between it and that shown in Plate 10.



During the past summer, Stomatopod larvae were met with at Fort Wool in the greatest abundance, two or three hundred being sometimes collected in a single evening. During the season there was a gradual increase in the size of the larvae; the smallest ones being most abundant in the early part of July, and very rarely met with in August; no large larvae were found in July, while the collections made in the first half of August consisted almost entirely of larvae in the later stages of development.

In a group where the development of closely allied forms is known to vary greatly, the only perfectly satisfactory method of investigation is by rearing a larva in confinement and watching its transformation into the adult. As I did not succeed in making the larvae thrive in confinement, I am not able to present such a series of observations upon one individual, and as our session at Fort Wool closed in the middle of August, before the larvae had entirely completed their metamorphosis, there is a bare possibility that they are not the young of *Squilla empusa*, but of some other Squilloid. The thousands of larvae which were collected, appeared to be specifically identical, however, and the series of forms, which were collected, was so complete, and the differences between the successive stages so slight, that there seems to be no reason to

doubt that they are all of the same species, and that species the only one which is known to occur in the Chesapeake Bay, *Squilla empusa*, (Say.)

The grotesque larva, Plate 10, appears, at first sight, to bear little resemblance to the full-grown animal, Plate 13, but the changes by which it is converted into the adult are very gradual, and can hardly be called a metamorphosis.

These changes are, an increase in the number of segments and appendages, and the alteration of the relative size of different parts, but each appendage has, at its first appearance, substantially the same form and relation as in the adult, and the changes of shape are very slight indeed, as compared with those of most podopthalmate Crustacea.

The youngest stage which was observed, is shown, magnified about 75 diameters, (Zeiss. Obj. A., Oc. 2, in Figures 1 and 2, Plate 9. Great numbers of young were found in this stage, and as no younger ones were met with, this is probably the form in which they escape from the eggs, and commence their larval life. The young at this stage, the "Alima," has most of the characteristics of a Decapod zoea. The eyes, 1, the antennules, 2, the antennae, 3, the mandibles, 4, and first and second maxillae, 5 and 6, are present, and have substantially the same form as the cor-

responding appendages of the adult. The first and second maxillipeds, 7 and 8, are quite large, and the second pair project from the sides of the carapace, and have the form of the grasping arms, Plate 12, 8\*, of the adult. The first and second maxillipeds differ from those of a Decapod zoea, since the exopodite is wanting, and the long jointed endopodite is not fringed with setae, and does not seem to be of much importance as an organ of locomotion.

The anterior half of the body is covered by a large zoea-like carapace, the anterior and posterior margins of which are prolonged into projecting spines. At the base of the long anterior spine, between the eye-stalks, on the ventral surface, there is a single small black ocellus, which should be shown in Figures 1 and 3 of Plate 9, just above the lower end of the line 1'.

This dot was so small in the original drawing that it has not been copied in the photo-electrotype, but it is shown in Plate 10, a little above the lower end of the line 0, and in Plate 11, Figure 1, at the end of the line 1.

The mid-body, or that organ which in the adult includes all the segments from the 9th to the 14th with their appendages, is here an elongated area without appendages, but showing, at its anterior end, a division into segments.

Following this is the flexible abdomen, terminating in a large telson, 20-21, which is preceded by five free abdominal segments, the first four of which carry swimming feet, represented only on one side of the figures.

The next stage which was figured is shown in Plate 9, Figure 3. This is a ventral view, drawn with the same magnifying power, 75 diameters, but somewhat reduced, so that the actual increase in size is greater than that shown in the figure. No larvae were found between this stage and that shown in Figure 1, and the change is probably brought about during a single moult.

In Figure 3 the mid-body is divided into its full number of six segments, 9-14, which are of about equal size, and none of them are furnished with appendages. As in the preceding stage, the abdomen consists of five segments, with appendages upon the first four, and a telson.

The next stage figured, Plate 10, was also drawn with a magnifying power of 75 diameters, but it is still more reduced than Figure 3, Plate 9. It also is a ventral view. A number of larvae were found between these two, and the interval between them represents at least two moults. The carapace and telson have undergone considerable change of form, and the ocular segment, 0, has become marked off at the anterior end of the body. The appendages of the six seg-

ments of the mid-body are now visible as minute buds, 9\*, 10\*, 11\*, 12\*, 13\*, 14\*, projecting from the ventral surfaces of these segments.

The first and second of these segments, the ninth and tenth from the anterior end of the body, are much more narrow than the others, and their appendages point outwards. The fifth abdominal segment, 19, is now furnished with a very small pair of swimming feet, 19\*, and the ganglion of the sixth abdominal segment, 20, has made its appearance, although the segment itself is not yet formed.

The next stage which was figured, Plate 11, Figure 1, was drawn with a magnifying power of about 30 diameters, and the drawing was more reduced than in Plate 10. The actual increase in size is therefore considerably more than twice that shown by the figures. Intermediate forms were collected, and several moults probably occur between the two stages figured. A figure, by Emmerton, of a stage which is probably one moult earlier than Plate 11, Figure 1, accompanies Smith's paper, already referred to, (U. S. Fish Commission Report, 1871 and 1872, Plate VIII, Fig. 36, No. 519.) In Plate 11, Figure 1, the carapace and telson have undergone considerable change of shape: the antennular segment, 2, has separated from the anterior end of the body; the first three segments of the mid-body, 9, 10 and 11,

have become crowded together; their ganglia are in contact with those of the preceding segments, and their appendages are chelate, and begin to twist forwards between the bases of the large grasping limbs. The sixth abdominal segment, 20, has separated from the telson, 21, and carries a pair of appendages, 20\*, which are larger than those of the preceding segments, and have substantially the same form as the large swimmerets of the adult. Plates 12 and 13, 20\*.

Older stages than this were examined and drawn, but as the changes, which occupy several moults, are very slight and consist mainly in a gradual approximation to the shape and proportions of the adult, they have not been engraved. Soon after the stage shown in Plate 11, Figure 1, the antennary segment separates from the anterior end of the body, so that we have, as in the adult, three free segments. Plate 11, Figure 3; 1, 2, 3; and Plates 12 and 13, *o. p* and *q*; in front of the carapace. In the adult the 9th, 10th and 11th segments, that is, the 1st, 2d and 3d of the mid-body, are crowded together, and their chelate appendages, Plate 12, 9\*, 10\* and 11\*, are directed forwards and lie below the mouth parts.

I will now describe the various stages of development, more in detail, and as all my notes were in the form of sketches, this description will be little more than an account of the figures.

## THE CARAPACE.

In the youngest larva, Figures 1 and 2, Plate 9, the carapace, including the rostrum, covers a little more than one-half the length of the body. The rostrum, *d*, is about half as long as the body of the carapace, and a little behind the point where it joins the carapace are a pair of small spines pointing forwards and outwards, which I shall call the *antero-lateral* spines, Figure 1, *e*. The margins of the carapace in front of these spines, what may be called the *antero-lateral* margins, meet at the base of the rostrum in an acute angle. At the outer angles of the posterior margin of the carapace are a pair of large spines, *f*, about as long as the rostrum, which I shall call the *postero-lateral* spines. The margin of the carapace between the antero- and postero-lateral spines, the *lateral* margin, is symmetrically curved, so that the middle of the carapace is much wider than the ends. The posterior margin of the carapace is deeply notched on the median line, where it passes over the body, and on its upper surface there is a short *dorsal* spine, *i*, Figure 2. At this stage the posterior margin of the carapace crosses the body at a point which corresponds to the constriction between the 13th and 14th segments of an older larva.

In the second stage, Figure 3, the carapace is much longer, and with the rostrum now makes three-fifths of the length of the body; the posterior margin still lies above the constriction between the 13th and 14th segments, and the increase in length is therefore due to the growth of the anterior half of the body. The various spines are of about the same relative size as at an earlier stage, but the antero-lateral margins of the carapace now make an obtuse angle with each other at the base of the rostrum, and the lateral margins are nearly straight and parallel. The outline of the carapace is accordingly rectangular instead of oval.

In the next stage figured, Plate 10, the carapace extends backwards only to the middle of the 13th segment, but notwithstanding this, its length is greatly increased, and with the rostrum it now forms two-thirds of the length of the body. Most of this increase is in the rostrum, which is now as long as the body of the carapace, instead of only half as long. The antero- and postero-lateral spines are also much longer than at an earlier stage, and the postero-lateral spines are slightly curved, but the curvature is probably due to the pressure of the cover glass which I was compelled to use to keep this larva in a proper position for drawing. All the drawings were made from living specimens, and it was especially difficult to keep



those at this stage quiet, or on their backs. The antero-lateral margins now meet the greatly enlarged base of the rostrum at quite an obtuse angle, and the nearly straight lateral margins diverge from each other, so that the space between the bases of the postero-lateral spines is almost twice as wide as that between the antero-lateral spines.

In the next stage the posterior margin of the carapace reaches only to the middle of the 12th segment, and the outline is substantially as before; the rostrum is still about as long as the body of the carapace, but in a ventral view the base is hidden by the ocular and antennary segments. In the adult the antero-lateral spines, Plate 13, *e*, are quite small, and all the others are wanting, unless a movable plate, *r*, represents the rostrum. The antero-lateral margins now meet at an angle of  $180^{\circ}$  and form the anterior face of the carapace. The lateral margins are nearly straight, and diverge from each other a little; and the postero-lateral angles, *f*, are now rounded.

#### THE SEGMENTS.

The time of appearance of the various segments and their appendages is shown in the accompanying table, pages 160 and 161, in which

APPENDAGES OF ADULTS.	NUMBER OF SEGMENT.	FIRST STAGE.	SECOND STAGE.
Eyes.	1	Appendages present, segments fused.	Same as in first stage.
1st Antennae.	2		
2d Antennae.	3		
Mandibles.	4		
1st Maxillae.	5		
2d Maxillae.	6		
Oral Palpus, or 1st Maxilliped.	7	Distinct. _____	Same as in first stage.
Grasping Hand, or 2d Maxilliped.	8	Distinct. _____	Same as in first stage.
1st Oral Hand, or 3d Maxilliped.	9	Segments distinct, but without appendages	Same as in first stage.
2d Oral Hand, or 1st Pereopod.	10		
3d Oral Hand, or 2d Pereopod.	11		
1st Walking Limb, or 3d Pereopod.	12	Represented by an un- segmented region.	Segments dis- tinct without appendages
2d Walking Limb, or 4th Pereopod.	13		
3d Walking Limb, or 5th Pereopod.	14		
1st Swimming Foot.	15	Segments distinct, with appendages.	Same as in first stage.
2d Swimming Foot.	16		
3d Swimming Foot.	17		
4th Swimming Foot.	18		
5th Swimming Foot.	19	Distinct without appen- dages.	Same as in first stage.
Swimmeret.	20	Fused.	Same as in first stage.
Telson.	21		

NUMBER OF SEGMENT.	THIRD STAGE.	FOURTH STAGE.	ADULT.
1	Segment <u>distinct.</u>	Same as in <u>third stage.</u>	Same as in <u>third stage.</u>
2	} Same as in first stage.	Segment <u>distinct.</u>	Same as in <u>fourth stage.</u>
3		} Same as in first stage.	Segment <u>distinct.</u>
4			} Same as in first stage.
5			
6			
7	Same as in <u>first stage.</u>	Same as in <u>first stage.</u>	Same as in <u>first stage.</u>
8	Same as in <u>first stage.</u>	Same as in <u>first stage.</u>	Same as in <u>first stage.</u>
9	} Appendages present as buds. Segments <u>distinct.</u>	Segments fused, limbs bending forward and chelate.	} Same as in fourth stage.
10		} Appendages present, segments <u>distinct.</u>	
11			
12			
13		} Same as in fourth stage.	
14			
15			
16	} Appendages present.	} Same as in third stage.	} Same as in fourth stage.
17			
18			
19			
20			
21	} Segment represented by its ganglion.	Segment and appendages present. Telson.	Same as in fourth stage.

the first column describes in ordinary type, the function of the appendage of the adult, and also gives in italics the corresponding appendages of an ordinary Decapod; the second gives the number of the segment, counting from the anterior end of the body of the adult; the third, the condition of the segment and its appendages, at the stage shown in Plate 1, Figure 1; the fourth, their condition at the stage shown in Plate 1, Figure 3; the fifth, their condition at the stage of Plate 2; the sixth, their condition at the stage shown in Plate 3, Figure 1, and the seventh, their condition in the adult. In the drawings the segments are indicated by figures, their appendages by figures with an \*, and their ganglia by figures with an accent.

From this table it will be seen that the free segments make their appearance in the following order, the antennary segment being the last: 9th, 10th, 11th, 15th, 16th, 17th, 18th, 19th, 12th, 13th, 14th, 1st, 20th, 21st, 2d, 3d; and it will also be seen that all the segments, except those which carry the mandibles and first and second maxillae, that is the 4th, 5th and 6th, are at some stage of development free from the carapace. As there is no reason to doubt that the carapace of *Squilla* is homologous with that of the Decapod zoea, and this again with that of the adult, the view that the Decapod carapace represents the terga of all the segments of the cephalothorax is clearly untenable.

## THE APPENDAGES.

*The eyes*, 1, are substantially like those of the adult, from the earliest stages.

*The antennules*. During the first and second stages the antennules, 2, are almost as long as the rostrum, and consist of a based three-jointed protopodite, which carries a short thick exopodite, *b*, with three setae at its tip, and a more slender endopodite, *a*, with two terminal setae. On the dorsal surface of the proximal end of the basal joint of the prodopodite is the spherical auditory sac, shown in the dorsal view, Figure 2. In the third stage, Plate 10, the exopodite, *b*, is divided into a very small terminal joint with one seta, and a long swollen joint with five; while the endopodite, *a*, is divided into three joints, each of which carries a seta. At this stage the antennules are much shorter than the rostrum. In the fourth stage, Plate 11, Figure 1, the antennules are longer than the rostrum; the exopodite is divided into two branches; the organ is carried upon a distinct segment, and is substantially like that of the adult.

*The antennae*. In the first stage, Plate 1, Figure 2, this, 3\*, consists of a three-jointed stem, with a terminal scale, which bears a few setae. In the fourth stage, Plate 11, Figure 1, the scale is large,

fringed with stout setae, and a three-jointed flagellum, *s*, springs from the suture between the first and second joints of the stem. The differences between this stage and the adult, will be seen by a comparison with Plate 12.

*The mouth parts* were not carefully studied, but three pairs, 4, 5, 6, and an upper lip, *n*, were present at all stages.

*The oral palpi.* These appendages are relatively much longer, Plate 9, Figures 1 and 2, 7, and much more movable at an early stage than in the adult; they consist of a long slender four-jointed stem, and a terminal joint furnished with hairs. During the earlier stages this terminal portion is only slightly bent upon the stem, but in the third and fourth stages, Plates 10 and 11, 7, it is so twisted as to lie parallel to the stem, and the stems themselves are now nearly straight, very slightly movable, and lie on the sides of the mouth parts, as in the adult.

*The grasping legs.* At the first stage these appendages, 8, have their characteristic adult form, and undergo little change. In the oldest larva which was captured, the teeth upon the terminal joint had not made their appearance. At the base of this appendage, during all the larval stages, there is a large flat membranous oval plate, *c*, which moves with the limb, and may perhaps be homologous with the gill of a Decapod.

*The oral hands.* These appendages are acquired between the stages shown in Plates 10 and 11. At first they project at right angles to the body, but they soon twist forwards over the mouth, and their basal joints become crowded together as in the adult. Each of them is furnished with a small gill-like plate, Plate 11, Figure 2, *c*, *c'*, *c''*, like that upon the basal joint of the grasping hand.

*The three pairs of walking limbs* appear soon after the oral hands, and consist of a protopodite, an exopodite and an endopodite, as in the adult.

*The abdominal appendages* have substantially the adult form, from the time of their first appearance, and consist of a protopodite which is long and narrow in the earlier stages, and short and wide in the adult; a scale like oval exopodite which has a fringe of a few stiff setae in the younger stages, and numerous flexible setae in the adult. The endopodite is very similar in shape, but carries on its inner margin, during the later stages, a second small joint, which in the adult, is in contact, on the median line, with the corresponding part of the endopodite of the other side of the body.

#### THE TELSON

Is at first, Plate 9, Figure 1, 20, 21, elongated, nearly rectangular, and nearly twice as long as

wide. Its posterior margin is serrated, and slightly concave. In the next stage, Plate 9, Figure 3, it is oval, about one-third longer than wide, and the posterior margin is deeply concave. In the third stage, Plate 10, it is octagonal, almost as wide as long, and its angles carry prominent teeth, which point backwards. The serrated posterior margin is still concave, and regularly curved.

In the next stage, Plate 11, Figure 1, the anterior portion of the telson has separated as the sixth abdominal segment, 20. The true telson, 21, is now about as wide as long, ten sided, and the angles are toothed. The posterior margin is notched in the centre, and consists of two straight serrated edges, meeting at an acute angle. The postero-lateral edges are also serrated. The characteristics of the adult telson are sufficiently well shown in Plate 13, 21.

#### THE NERVOUS SYSTEM.

In the first stage the optic ganglia form a large rounded body, Plate 9, Figure 1, 1', imperfectly divided posteriorly into two lobes, by a longitudinal furrow, which runs forwards, about half way to the anterior margin of the mass. The two ganglia of the antennules, 2', are entirely



separated on the median line by the furrow, but are in contact with each other; well marked furrows separate them from the optic ganglia.

From the posterior margins of these ganglia a pair of very thick commissures, *g*, pass backwards, diverging from each other, and forming the sides of a large oval foramen, *h*. The ganglia of all the segments, from the 1st maxillae to the last pair of walking legs, 5 to 14, are represented by a long rod-like body on the median line, with its anterior end divided into eight ganglia, and the posterior portion undivided.

This rod is not divided into halves, but is continuous across the median line. The first of the ganglia is very small, while the second, that of the second maxillae, Figure 1, 6', is very much larger, the third, that of the oral palpi, 7', still larger, and the others about equal in size. In the mid-body the number of constrictions upon the nerve-rod is one greater than the number upon the surface of the body, and the ganglion of the 12th segment, 12', accordingly appears before the segment itself. In the abdomen there are five sharply defined ganglia, 15' to 19', one for each segment, and they are widely separated from each other, and connected by double commissures.

In the next stage, Plate 9, Figure 3, the cerebrum has undergone considerable change, and

now consist of a single anterior rounded mass, 1', the optic ganglia, and two posterior portions, separated on the median line, with a slight constriction dividing them into an anterior antennary ganglion, 2', and a posterior antennary ganglion, 3', which is sharply separated from the commissure, which is now a small thread-like fibre. Each of the segments of the mid-body is now furnished with a ganglion, 9 to 14', but they are still crowded together and without commissures.

In the next stage, Plate 10, the ganglia of the 12th, 13th and 14th segments are separated from each other, spherical, and connected by short double commissures. The ganglion of the sixth abdominal segment, 20', is present, but not the segment itself. The commissures between the abdominal ganglia are much longer than at an earlier stage.

In the next stage the ganglia of the 7th to 11th segments, Plate 11, Figures 1 and 2, 7 to 11, are crowded together and form a compact thoracic mass as in the adult. The ganglia of all the succeeding segments, 14 to 20', are compact, widely separated and connected by long slender double commissures, and the nervous system now has its adult form.

EXPLANATION OF THE FIGURES.

PLATE 9.

**FIGURE 1.**—Ventral view of youngest stage observed, magnified 75 diameters.

**FIGURE 2.**—Dorsal view of another larva in the same stage.

**FIGURE 3.**—Ventral view of an older larva.

- 1 to 21     Segments and appendages.
- 1' to 19'.   Ganglia.
- a.   Endopodite of antennule.
- b.   Exopodite of antennule.
- c.   Gill-like plate upon basal joint of Grasping Hand.
- d.   Rostrum.
- e.   Antero-lateral spines of carapace.
- f.   Postero-lateral spines of carapace.
- g.   Oesophageal nerve commissure.
- h.   Its foramen.
- i.   Dorsal spine of carapace.
- k.   Stomach.

PLATE 10.

**A** somewhat older larva, magnified 75 diameters; ventral view.

- 1   to 21.   Segments.
- 1\* to 19\*.   Appendages.
- 1'   to 20'.   Ganglia.

The small letters of reference as before.

PLATE 11.

**FIGURE 1.**—Still older larva, viewed from the ventral surface, and magnified 30 diameters.

**FIGURE 2** —Basal joints and ganglia of the appendages from the 7th to the 11th segments, magnified 75 diameters.

**FIGURE 3.**—Anterior end of carapace with proximal ends of appendages, magnified 75 diameters.

The letters and figures of reference are the same as in Plate 10.

## PLATE 12.

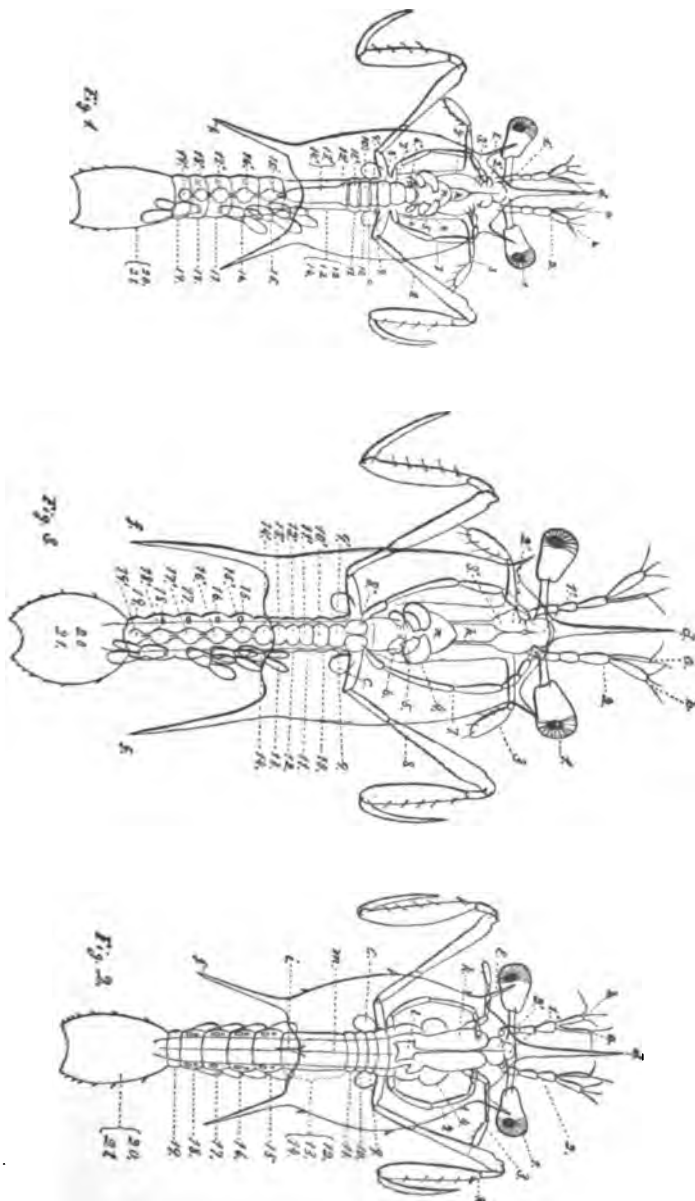
Ventral view of adult.

## PLATE 13.

Dorsal view of adult.

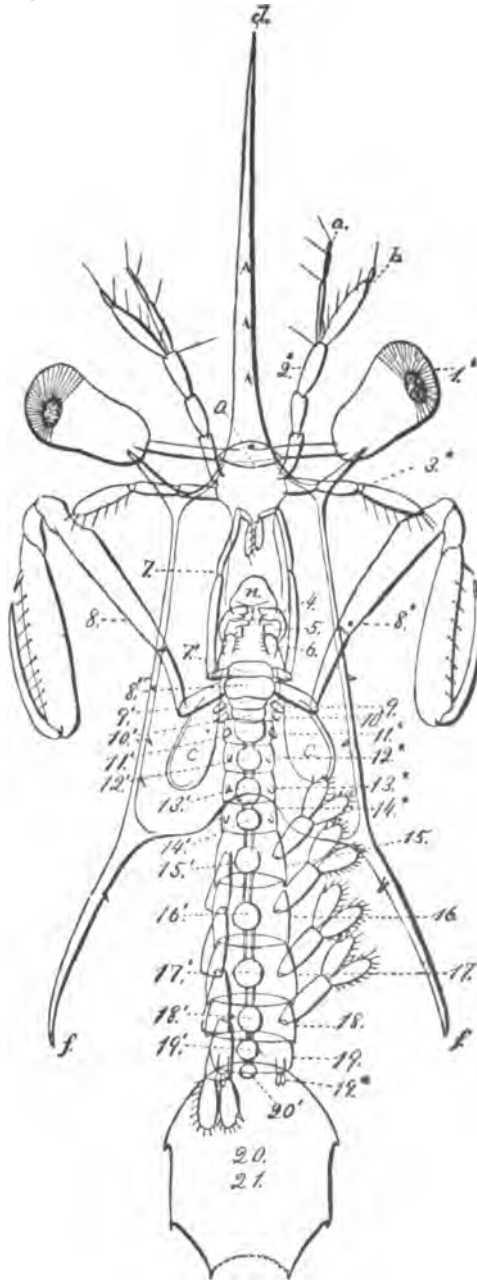
- o.* Optic segment.
- p.* Antennulary segment.
- q.* Antennary segment.
- br.* Gills.
- l.* Exopodite of swimmeret.
- u.* Spine of swimmeret.
- v.* Endopodite of swimmeret.
- w.* Anus.

The other letters and figures as in Plate 10.





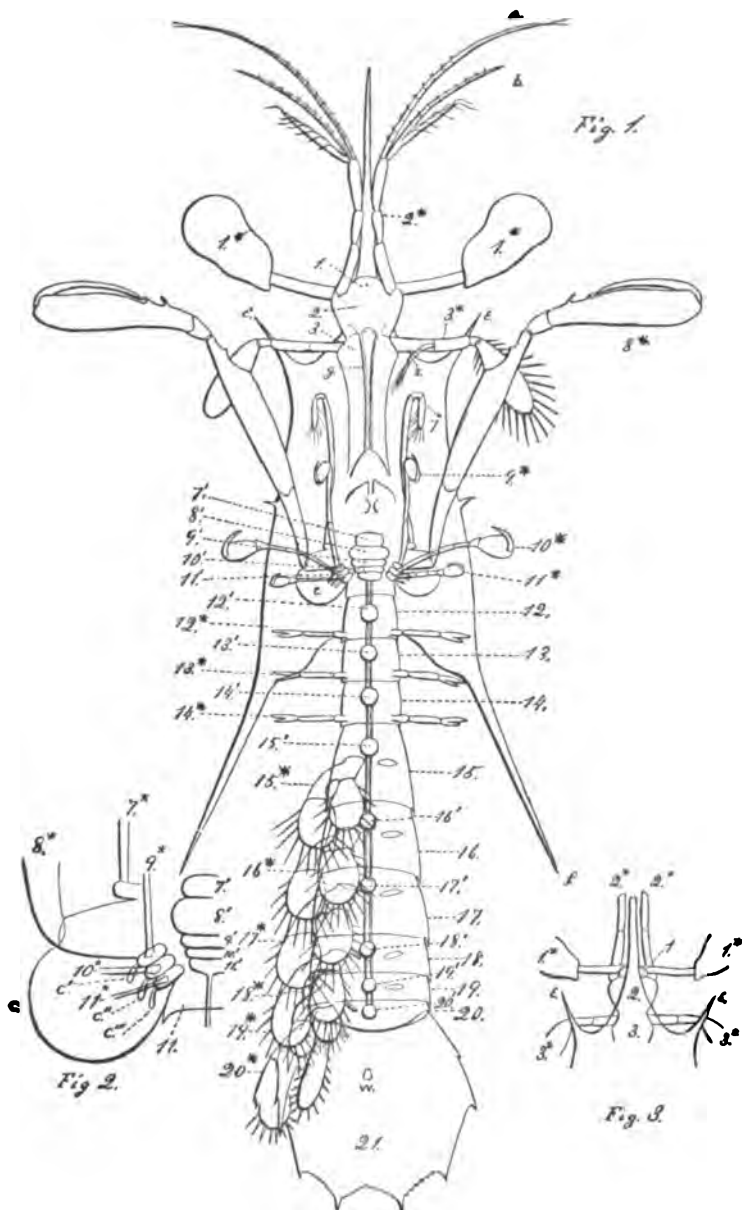
**Plate 10.**



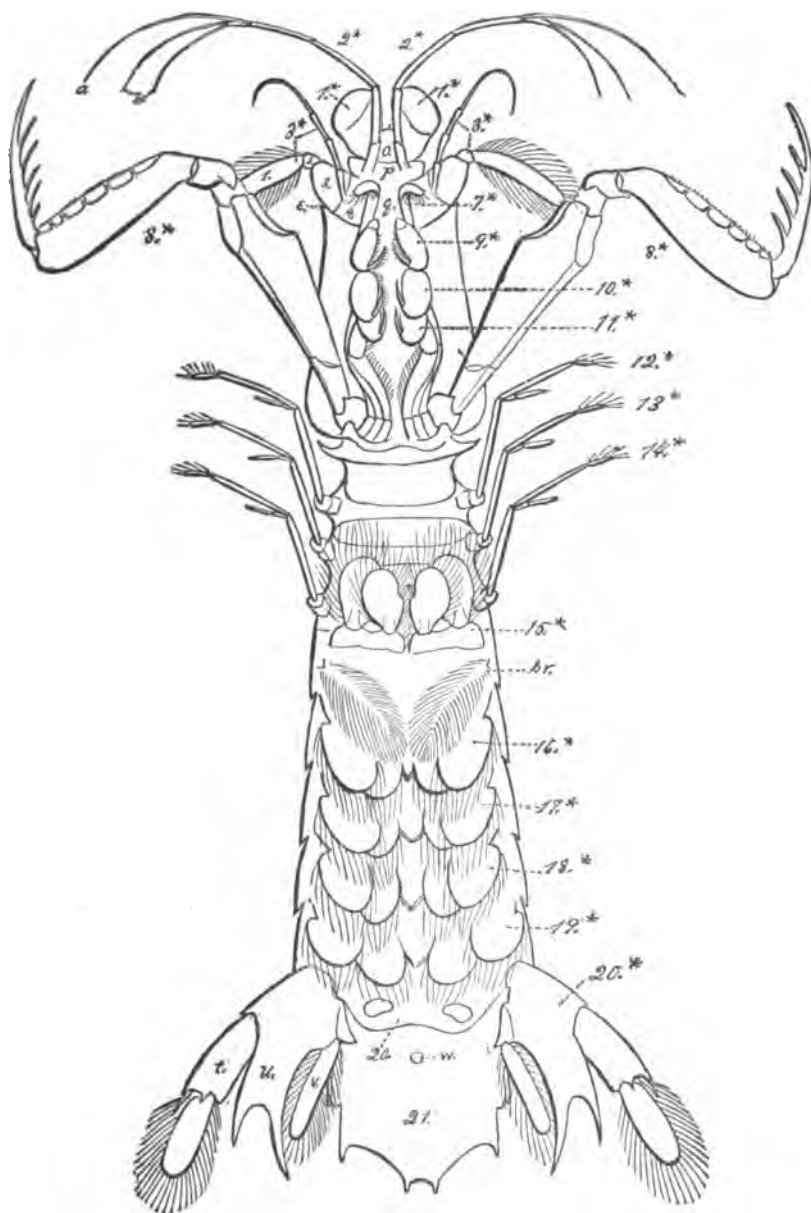
**W. K. BROOKS, DEL.**



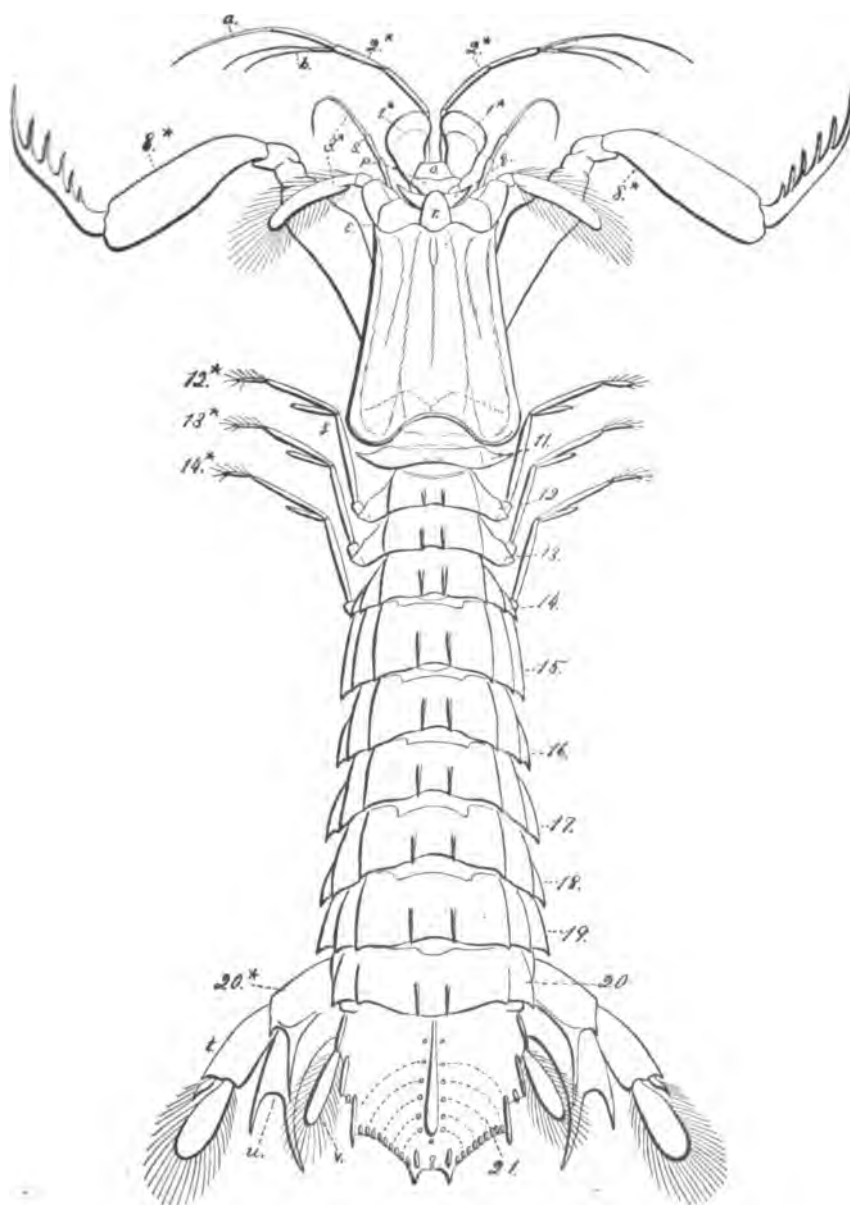














CONTRIBUTIONS FROM THE CHESAPEAKE ZOOLOGICAL LABORATORY.

JOHNS HOPKINS UNIVERSITY,

BALTIMORE.

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STUDIES

FROM THE

BIOLOGICAL LABORATORY.

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THE DEVELOPMENT OF THE OYSTER,

By W. K. BROOKS,

ASSOCIATE IN BIOLOGY.

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No. IV.

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**DEVELOPMENT OF THE AMERICAN OYSTER.**

**BY W. K. BROOKS.**

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[Reprinted from the Report of the Commissioners of Fisheries of Maryland, 1880.]

## DEVELOPMENT OF THE AMERICAN OYSTER,

(*Ostrea virginiana* List.)

BY W. K. BROOKS,

*Associate in Biology, Johns Hopkins University,*

BALTIMORE, MD.

At the request of Major Ferguson, Fish Commissioner of Maryland, that I should attempt to trace the development of the young oyster, I made arrangements which enabled me to leave Baltimore a month before the close of my year's work at the University, and the opening of the Seaside Laboratory, Dr. Martin and Dr. Sihler generously taking charge of my classes, and affording me an entire month for uninterrupted work upon the oyster. The United States Coast Survey having determined to continue the examination of the oyster beds of Pokamoke and Tangier Sounds, Major Ferguson was desirous of having the biological investigations commenced in the same locality I therefore arranged to open the Seaside Laboratory of the Johns Hopkins University in June, 1879, at Crisfield, within reach of the great natural oyster beds of Tangier and Pokamoke Sounds.

While I regard the information which I was able to obtain upon certain purely scientific questions in embryology as the most important and valuable result of my summer's work, I am aware that most of the persons who are interested in the habits of the oyster and in oyster culture would not care to read a purely technical embryological paper. It seems best, then, to divide my account into two parts, and to give first a somewhat popular description of the method of artificial fertilization, with a description of a sufficient number of my figures to convey a general idea of the manner of develop-

ment, and then to complete the paper in a second part, devoted to a minute description of the figures, a discussion of the theoretical and comparative bearings of my observations and a notice of the observations and views of others.

The place which was selected was excellently fitted for the work. The town of Crisfield, Md., is situated at the junction of Tangier and Pokomoke Sounds, two large and wide but shallow sheets of water, whose muddy bottoms abound in oysters of the best quality. The town is one of the most important centres of the oyster-packing industry, and is built in the water upon the shells of the oysters, which have been shipped to all parts of the country for consumption. As fast as the oysters are opened the shells are used to build up new land, and with them a large peninsula has been formed, stretching out for more than half a mile from the low marshy shore towards the oyster beds, and furnishing room for wide streets, a railroad and a steamboat landing, in addition to the large packing houses and the shops and dwellings for a population of several thousand people. A single view of the long, white, solid streets and docks of this singular town would convey a much more vivid idea of the oyster-packing industry than any number of tables of statistics.

I found everybody greatly interested in all that relates to the oyster, and ready to give me every help in my work, but I am especially indebted to Dr. H. H. Gunby, Mr. T. S. Hodson and Mr. J. J. Lawson for many kindly favors, which not only enabled me to work to the greatest advantage, but also rendered my stay among them very pleasant.

#### BREEDING HABITS OF THE AMERICAN OYSTER.

Our knowledge of the development of the oyster is derived from the fragmentary observations of various German, French, English and Russian embryologists, whose work will be noticed at length further on. While the subject has received the attention of a number of observers, no one has been able to get anything like a complete series of the early stages of development, and I approached my work without hope of ac-

·completing much of purely scientific value, although I did ·expect to obtain some information as to the time and conditions of spawning, and other questions of economic interest. My uncertainty of success was increased by the total failure of an attempt which I had made the summer before.

All the published papers upon the subject state that the ·eggs are fertilized inside the body of the parent, and that the young are carried inside the parent shell until they are quite well advanced in development, and provided with shells of their own; that they swim about after they are discharged from the parent until they find a place to attach themselves, but that they undergo no change of structure between the time when they leave the parent and the time when they become fixed. Misled by these statements, which are not true with our species, I opened numbers of oysters during the summer of 1878, and carefully examined the contents of the gills and mantle chambers, but found no young oysters. I concluded that the time during which the young are carried by the parent must be so short that I had missed it, and I entered upon the work this season with the determination to examine adult oysters every day, through the breeding season, in search of young, and at the same time to try to raise the young for myself by artificially fertilizing the eggs after I had removed them from the body of the parent.

I met with complete success with the second method from the beginning, and succeeded in raising countless millions of young oysters, and in tracing them through all their stages of development until they had acquired all the characteristics which the European embryologists have described and figured in the young of the European oyster at the time it leaves its parent to become fixed for life.

I reached Crisfield on the 19th of May, and established myself about three miles from the town and about half a mile from Pokamoke Sound, and on Monday, the 21st, I opened a dozen fresh oysters, and found three females with their ovaries filled with ripe ova, and one male with ripe spermatozoa.

I mixed the contents of the reproductive organs of these four oysters, and within two hours after the commencement

of my first experiment, I learned by the microscope that the attempt at artificial fertilization was successful, and that nearly all of my eggs had started on their long path towards the adult form.

I made careful microscopic examination of the gills and mantles of all these oysters, but neither at this time nor afterwards did I find any fertilized eggs or young inside the parent shell, although I examined more than a thousand adults during the season. During the summer I found females with the ovaries so distended with ripe eggs that they were oozing from the openings of the oviducts; others where the ovaries were half emptied, and others which had discharged almost all their eggs, and others at all the intermediate stages, but in no case did I find a single developing egg inside the shell of the parent.

I have accumulated enough evidence to show beyond the possibility of doubt, that so far as the oysters of the Chesapeake Bay, during the summer of 1879, are concerned, the eggs are fertilized outside the body of the parent, and that, during the period which the young European oyster passes inside the mantle cavity of its parent, the young of our oyster swims at large in the open ocean.

While this evidence cannot be regarded as sufficient to show that the young of the American oyster are never carried by their parents, it is certainly enough to show that this cannot be assumed from the analogy of the European oyster. Most of the popular treatises on the use of the microscope state that during the summer young oysters may be found inside the shells of the old ones, and as the number of amateur workers with the microscope in this country is quite large, I should be glad to learn whether any one has ever found this to be the case with American oysters.

Until some such evidence is produced it is fair to conclude that my results are to be applied to all the American oysters, and that there is a very important difference between them and the European species.



## ANATOMICAL OUTLINE SKETCH.

The thorough study of the anatomy of the adult oyster is rather difficult, but there is no difficulty in gaining all the knowledge which is needed for procuring and fertilizing the eggs. As I hope that a way will be found to turn my observations to practical account in oyster culture, I will give a very brief sketch of the structure of the oyster—such a sketch as will enable any one who reads it with an opened oyster before him to acquire the necessary anatomical knowledge. It is hardly possible to write such a description without using a few technical terms, such as anterior and posterior, dorsal and ventral. As the end of the body where the mouth is placed is not marked by a head, it must be spoken of as the *anterior end*, not as the *head*, and the opposite end as the *posterior*. As the oyster lies on one side, the *top* and *bottom* of its body do not correspond to the regions which occupy these positions in an upright mussel or clam, and it is most convenient to speak of that part of the oyster's body which answers to the upper surface of a clam as dorsal and the opposite as ventral.

The general structure of an oyster may be roughly represented by a long narrow memorandum book, with the back at one of the narrow ends instead of at one of the long ones. The covers of such a book represent the two shells of the oyster and the back represents the hinge, or the area where the two valves of the shell are fastened together by the hinge ligament. This ligament is an elastic, dark brown structure, which is placed in such a relation to the valves of the shell that it tends to throw their free ends a little apart. In order to understand its manner of working, open the memorandum book and place between its leaves, close to the back, a small piece of rubber to represent the ligament. If the free ends or the cover are pulled together the rubber will be compressed and will throw the covers apart as soon as they are loosened. The ligament of the oyster-shell tends by its elasticity to keep the shell open at all times, and while the oyster is lying un-

disturbed upon the bottom, or when its muscle is cut, or when the animal is dying or dead, the edges of the shell are separated a little.

The shell is lined by a thin membrane, the mantle, which folds down on each side, and may be compared to the leaf next the cover on each side of the book. The next two leaves of each side roughly represent the four gills, the so-called "beard" of the oyster, which hang down like leaves into the space inside the two lobes of the mantle. The remaining leaves may be compared to the body or *visceral mass* of the oyster.

Although the oyster lies upon the bottom with one shell above and one below, the shells are not upon the top and bottom of the body, but upon the right and the left sides. The two shells are symmetrical in the young oyster, but after it becomes attached the lower or attached side grows faster than the other, and becomes deep and spoon-shaped, while the free valve remains nearly flat. In nearly every case, the lower or deep valve is the left. As the hinge marks the anterior end of the body, an oyster which is held on edge with the hinge away from the observer and the flat valve on the right side, will be placed with its dorsal surface uppermost, its ventral surface below, its anterior end away from the observer, and its posterior end towards him, and its right and left sides on his right and left hands respectively.

In order to examine the soft parts, the oyster should be opened by gently working a thin flat knife blade under the posterior end of the right valve of the shell, and pushing the blade forwards until it strikes and cuts the strong adductor muscle, which passes from one shell to another and pulls them together. As soon as this muscle is cut the valves separate a little, and the right valve may be raised up and broken off from the left, thus exposing the right side of the body. The surface of the body is covered by the mantle, a thin membrane which is attached to the body over a great part of its surface, but hangs free like a curtain around nearly the whole circumference. By raising its edge, or gently tearing the whole right half away from the body, the gills will be exposed.

These are four parallel plates which occupy the ventral half of the mantle cavity and extend from the posterior nearly to the anterior end of the body. Their ventral edges are free, but their dorsal edges are united to each other, to the mantle and to the body. The space above or dorsal to the posterior ends of gills, is occupied by the oval, firm, adductor muscle, the so-called "heart." For some time I was at a loss to know how the muscle came to be called the heart, but a friend told me that he had always supposed that this was the heart, since the oyster dies when it is injured. The supposed "death" is simply the opening of the shell when the animal loses the power to keep it shut. Between this muscle and the hinge the space above the gills is occupied by the body, or *visceral mass*, which is made up mainly of the light colored reproductive organs and the dark colored digestive organs, packed together in one continuous mass.

If the oyster has been opened very carefully, a transparent crescent-shaped space will be seen between the muscle and the visceral mass. This space is the pericardium, and if the delicate membrane which forms its sides be carefully cut away the heart may be found without any difficulty, lying in this cavity, and pulsating slowly. If the oyster has been opened roughly, or if it has been out of water for some time, the rate of beating may be as low as one a minute, or even less, so the heart must be watched attentively for some time in order to see one of the contractions.

The heart is made up of two chambers, a loose spongy transparent *auricle*, which occupies the lower part of the pericardium, and receives blood from the gills through transparent blood vessels, which may usually be seen without difficulty running from the gills towards the heart, and a more compact white *ventricle*, which drives the blood out of the pericardium through transparent arteries, which are usually quite conspicuous.

The visceral mass is prolonged backwards over the pericardium and the adductor muscles, and here contains the rectum surrounded by prolongations of the white reproductive or-

gans. Still farther back, on the middle of the posterior face of the adductor muscle, is the anus, a long vertical slit, opening into the space between the lobes of the mantle and above the posterior ends of the gills.

In front of the gills, that is between them and the hinge, there are four fleshy flaps—the lips—two on each side of the body. They are much like the gills in appearance, and they are connected with each other by two ridges which run across the middle of the body close to the anterior end, and between these folds is the large oval mouth, which is thus seen to be situated, not at the open end of the shell, but as far away from it as possible. As the oyster is immovably fixed upon the bottom, and has no arms or other structures for seizing food and carrying it to the mouth, the question how it obtains its food at once suggests itself. If a fragment of one of the gills is examined with a microscope, it will be found to be covered with very small hairs, or *cilia*, arranged in rows. Each of these cilia is constantly swinging back and forth, with a motion something like that of an oar in rowing. The motion is quick and strong in one direction and slower in the other. As all the cilia of a row swing together, they act like a line of oars, only they are fastened to the gill, and as this is immovable, they do not move forwards through the water, but produce a current of water in the opposite direction. This action is not directed by the animal, for it can be observed for hours in a fragment cut out of the gill, and if such a fragment be supplied with fresh sea water, the motion will continue until it begins to decay. While the oyster lies undisturbed on the bottom, with its muscle relaxed and its shell open, the sea water is drawn on to the gills by the action of the cilia, for although each cilium is too small to be seen without a microscope, they cover the gills in such great numbers that their united action produces quite a vigorous stream of water, which is drawn through the shell and is then forced through very small openings on the surfaces of the gills into the *water tubes*, inside the gills, and through these tubes into the mantle cavity, and so out of the

shell again. As the stream of water passes through the gills the blood is aerated by contact with it. The food of the oyster consists entirely of minute animal and vegetable organisms and small particles of organized matter. Ordinary sea water contains an abundance of this sort of food, which is drawn into the gills with the water, but as the water strains through the pores into the water tubes, the food particles are caught on the surface of the gills by a layer of adhesive slime which covers all the soft parts of the body. As soon as they are entangled the cilia strike against them in such a way as to roll or slide them along the gills towards the mouth. When they reach the anterior ends of the gills they are pushed off and fall between the lips, and these again are covered with cilia, which carry the particles forwards until they slide into the mouth, which is always wide open and ciliated, so as to draw the food through the œsophagus into the stomach. Whenever the shell is open these cilia are in action, and as long as the oyster is breathing a current of food is sliding into its mouth.

The cilia and particles of food are too small to be seen without a microscope, but if finely powdered carmine be sprinkled over the gills of a fresh oyster, which has been carefully opened and placed in a shallow dish of sea water, careful observation will show that as soon as the colored particles touch the gills they begin to slide along with a motion which is quite uniform, but not much faster than that of the minute hand of a watch.

This slow, steady, gliding motion, without any visible cause, is a very striking sight, and with a little care the particles may be followed up to and into the mouth.

In order to trace the course of the digestive organs, the visceral mass may be split with a sharp knife or razor. If the split is pretty near the middle of the body, each half will show sections of the short, folded œsophagus, running upwards from the mouth, and the irregular stomach, with thick semi-transparent walls, surrounded by the compact, dark

greenish liver. Back of the liver and stomach the convoluted intestine will be seen, cut irregularly at several points by the section.

The coils of the intestine are imbedded in a light-colored mass of tissue—the reproductive organ—which forms the greater part of the visceral mass. The reproductive organ varies greatly according to the season, and forms most of what is known as the “fat” of the oyster.

There are no accessory organs of reproduction, and the position, form and general appearance of the reproductive organ is the same in both sexes. There is no characteristic by which a male oyster can be distinguished from a female without microscopic examination. As the reproductive organ has an opening on each side of the body, it is usually spoken of as double, but in the adult oyster it forms one continuous mass, with no trace of a division into halves, and extends entirely across the body and into all the bends and folds of the digestive tract.

As my observations only extend over one summer, I cannot make any general statements as to the breeding season, except that the oysters in shallow water spawn first, and those in deeper water later, as the water becomes warmer. Nearly all the oysters in shallow water spawn at about the same time, but there is more difference in the oysters taken from the same bed in deep water. Oysters in from one to six feet of water in the vicinity of Crisfield, probably spawn between the middle and end of May, but oysters with ripe eggs were found in water from five to six fathoms deep from the 1st to the 30th of July, although most of them spawn late in June.

#### ARTIFICIAL IMPREGNATION OF THE OYSTER EGGS.

If a number of oysters are opened during the breeding season, a few will be found with the reproductive organ greatly distended and of an uniform pure opaque white color. These are oysters which are spawning or nearly ready to spawn.

If the point of a knife be pushed into the reproductive organ a milk-like fluid will ooze out of the cut, and a little of it may be taken up on a knife blade and transferred to a glass slide for examination. The drop of fluid should be thoroughly mixed with a drop of sea water and placed on the slide, and gently covered with a cover-glass, and examined with a magnifying power of about one hundred diameters. If the specimen is a female, this power will show that the white fluid is almost entirely made up of irregular pear-shaped ovarian eggs (Figure 49), each of which contains a large circular transparent germinative vesicle surrounded by a layer of granular slightly opaque yolk. It is almost impossible to describe the slight differences which distinguish the perfectly ripe egg from those which are nearly ripe but not capable of fertilization, although a very little experience will enable one to tell whether it is worth while to attempt the fertilization of the eggs of any given female.

When the drop of fluid is thoroughly mixed with the sea water, the eggs should appear clean, sharply defined, separate from each other, and pretty uniformly distributed through the drop, as shown in the figure. If they adhere to each other, or if their outlines are indistinct, or if there is much fine granular matter scattered between the eggs, it is probable that the attempt at artificial fertilization will at best be only partially successful.

When a perfectly ripe female is found, it should be set aside and the search continued for a male. The question of the sex of the oyster has long been a matter of dispute, and the subject will be fully discussed in another place. All that concerns us now is to know that for all practical purposes the sexes are separate in the European as well as the American oyster. At the breeding season each individual is either exclusively a male or exclusively a female. Out of several thousand which I examined, I have not found one which contained both eggs and male cells, and all the best authorities upon the European oyster make the same statement, although there is some reason for the belief that an oyster may give

rise to eggs one season and to male cells another year. When a drop of the milky fluid from a ripe male is mixed with a little sea water and examined with a magnifying power of one hundred diameters, it is seen at a glance to be quite different from the fluid of a female. There are no large bodies like the eggs, but the fluid is filled with innumerable numbers of minute granules (Figure 48), which are so small that they are barely visible when magnified one hundred diameters. They are not uniformly distributed, but are much more numerous at some points than at others, and for this reason the fluid has a cloudy or curdled appearance. By selecting a place where the granules are few and pretty well scattered, very careful watching will show that each of them has a lively dancing motion, and examination with a power of five hundred diameters will show that each of them is tad-poll-shaped (Figure 50), and consists of a small, oval, sharply defined "head" and a long, delicate "tail," by the lashing of which the dancing is produced.

It is more difficult to decide whether the male cells are perfectly ripe than it is to decide in the case of the eggs. With a magnifying power of five hundred diameters, each "head" should have a clear, well-marked outline, and they should be very uniform in size, and separated from each other, as in Figure 50. Under very favorable circumstances this power should also show the "tails," as very faint undulating lines.

If the "heads" vary much in size, or if they are aggregated into bunches, with the "tails" radiating from the bunches in all directions, or if there is much granular matter so small that the outlines of the particles are not visible when magnified five hundred diameters, the fluid is not perfectly ripe, and fertilization with it will not in all probability be very successful.

#### NUMBER OF EGGS.

As the male cells are infinitely more numerous than the eggs, the ripe fluid from even one small male is enough to fertilize all the eggs of five or six large females.



The number of male cells which a single male will yield is great beyond all power of expression, but the number of eggs which an average female will furnish may be estimated with sufficient exactness. A single ripe egg measures about one-five hundredth of an inch in diameter, or five hundred laid in a row, touching each other, would make one inch; and a square-inch would contain five hundred such rows, or  $500 \times 500 = 250,000$  eggs. Nearly all the eggs of a perfectly ripe female may be washed out of the ovary into a beaker of sea water, and as they are heavier than the sea water, they soon sink to the bottom, and the eggs of a medium sized female will cover the bottom of a beaker two inches in diameter with a layer of eggs one-twentieth of an inch deep. The area of the bottom of a beaker two inches in diameter is a little more than three square inches, and a layer of eggs one-twentieth of an inch deep, covering three square inches, is equal to one three-twentieths of an inch deep and two square, and as a single layer of eggs is one-five-hundredth of an inch thick, a layer three-twentieths of an inch thick will contain seventy-five layers of eggs, with 250,000 eggs in each layer, or 18,750,000 eggs. It is difficult to get the eggs perfectly pure, and if we allow one-half for foreign matter and errors of measurement, and for imperfect contact between the eggs, we shall have more than nine millions as the number of eggs laid by an oyster of average size, a number which is probably less than the true number.

Möbius estimates the number of eggs laid by an average European oyster at 1,012,925, or only one-ninth the number laid by an ordinary American oyster, but the American oyster is very much larger than the European, while its eggs are less than one-third as large, so the want of agreement between these estimates does not indicate that either of them is incorrect.\* Another estimate of the number of eggs

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\*Möbius' measurement, from .15 to .18 millimeters, is given (Austern und Austern-wirtschaft, 1877), as the diameter, not of the egg, but of the embryo, but his figures show that the European oyster, like the American, does not grow much during the early stages of development, but remains of about the same size as the egg.

laid by the European oyster is given by Eyton, (*History of the Oyster and Oyster Fisheries*, by T. C. Eyton. London: 1858). He says, p. 24, that there are about 1,800,000, and therefore agrees pretty closely with Möbius.

An unusually large American oyster will yield nearly a cubic inch of eggs, and if these were all in absolute contact with each other, and there were no portions of the ovaries or other organs mixed with them, the cubic inch would contain 500<sup>3</sup>, or 125,000,000. Dividing this, as before, by two, to allow for foreign matter, interspaces and errors of measurement, we have about 60,000,000 as the possible number of eggs from a single oyster.

Although each male contains enough fluid to fertilize the eggs of several females, there does not seem to be much difference in the number of individuals of the two sexes. When a dozen oysters are opened and examined, there may be five or six ripe females and no males, but in another case a dozen oysters may furnish several ripe males but no females, and in the long run the sexes seem to be about equally numerous. Oystermen believe that the male may be distinguished from the female by certain characteristics, such as the presence of black pigment in the mantle, but microscopic examination shows that these marks have no such meaning, and that there are no differences between the sexes except the microscopic ones. It is not necessary to use the microscope in every case, however, for a little experience will enable a sharp observer to recognize a ripe female without the microscope. If a little of the milky fluid from the ovary of a female with ripe or nearly ripe eggs, be taken upon the point of a clean, bright knife blade, and allowed to flow over it in a thin film, a sharp eye can barely detect the eggs as white dots, while the male fluid appears perfectly homogeneous under the same circumstances, as do the contents of the ovary of an immature female, or one which has finished spawning. When the eggs are mixed with a drop of water, they can be diffused through it without difficulty, while the male fluid is more adhesive and difficult to mix with the water. By these indications, I was

able in nearly every case to judge of the sex of the oyster before I had made use of the microscope.

In order to fertilize the eggs, all that is necessary is the mixture of the ripe eggs with a little of the ripe male fluid in a drop of water. If the point of a knife-blade be dipped in the fluid from a female and touched to a glass slide, and then dipped into the fluid of a male and touched to the same part of the slide, and a drop of sea water be added, to cause the two to meet, most of the eggs will be fertilized, and their early stages of development can be studied in a single drop of water, but to secure the fertilization and healthy development of large numbers of eggs, several precautions are necessary, as well as a few instruments and pieces of apparatus.

The following is a list of the things needed for procuring, fertilizing and hatching the eggs: A pair of sharp-pointed scissors; a pair of small forceps; half a dozen watch crystals; a set of about half a dozen glass beakers, or tumblers, of different sizes, from half a pint up to half a gallon; two or three dipping tubes, or glass tubes six or eight inches long, open at both ends, but with one end drawn out to a fine point; a small glass or rubber siphon for drawing the water out of the beakers. For tracing the development of the eggs, a microscope, magnifying at least one hundred diameters, and half a dozen glass slides and thin glass covers are wanted.

After the oysters have been opened, and at least one ripe male and one ripe female found, cut off the mantle lobes and gills of the male with the scissors, close to the visceral mass, and tear them out with the forceps and throw them away. Cut around the adductor muscle with the scissors, so that the visceral mass may be lifted out of the shell and transferred to a small saucer or to a watch crystal. Holding the visceral mass with the forceps, cut out with the scissors as much as possible of the digestive organs and liver and throw them away, and then chop up the reproductive organs with the scissors, picking out and throwing away any fragments of the liver, digestive organs, mantle or gills which may present themselves. In order to have the young oyster thrive, the

water must be kept free from fragments of the various organs of the adult, as these would soon decay and destroy the embryos, and it is therefore important to remove them as completely as possible. After the mass has been chopped up as fine as possible, fill up the watch crystal with fresh sea water, stir it up, and then allow it to run into one of the smallest beakers, which has been nearly filled with sea water. As the water runs out of the watch crystal, be careful to allow as few of the fragments as possible to run with it.

Now fill up the watch crystal with water again, and stir and pour off as before, and repeat the process until nearly all of the male fluid has been washed out of the fragments and poured into the beaker. Stir the contents of the beaker for a short time, and then allow it to stand about five minutes to allow any fragments to settle to the bottom, then pour the fluid which should be quite milky, into another small beaker, leaving behind to be thrown away, any particles which may have settled to the bottom. The male cells retain their full vitality for several hours after they have been mixed with sea water, so the beaker may be set aside to wait until the eggs are ready. The eggs swell up and break to pieces within a very few minutes after they are mixed with water, unless they are fertilized at once, so it is much better to add the eggs to a previously prepared mixture of male cells and water than it is to put the eggs into the water to wait until the male fluid is got ready.

Taking now one of the females, remove and chop up the ovary in the same way in another watch crystal, observing the same precautions in removing all portions of the body. Fill the watch glass with water, and stir and pour off into the beaker as before, giving the contents of the beaker a good stirring after each lot of eggs is added, in order to diffuse them through the water at once, and thus insure the speedy contact of each of them with some of the male cells.

Fill the crystal with water again, and stir and pour off, and repeat until all the eggs have been washed out of the fragments of the ovary.

Another female may now be cut up, and the eggs may be added to the contents of the same beaker, but if the females are large, and yield many eggs, it is not best to use more than one, for although there are enough male cells to fertilize a very great number of eggs, the eggs are heavier than water and soon sink to the bottom, and if they form a very thick layer, only those which lie near the surface have room to develop.

The beaker should now be allowed to stand for about ten minutes, and in the meantime some of the eggs may be picked out with a dipping tube for examination under the microscope. In using the dipping tube, cover the large end with the tip of the finger, and run the small end down close to the bottom of the beaker, and then take the finger off the top, and as the water runs in at the bottom it will carry some of the eggs with it. When the tube is filled, place the finger on the top again, and draw it out of the water, and, holding it perpendicularly on the centre of a glass slide, and taking the finger off the top, allow a good sized drop to run out into the slide.

If things are working properly, each egg should now have a number of male cells attached by their heads to its outer surface, with their tails radiating from it in all directions, as shown in Figure 51.

It is not necessary that more than one male cell should fasten onto each egg, but they usually cover them in such numbers that the lashing of their tails causes the eggs to rotate and move through the water.

As soon as all the eggs have male cells attached to them, it is necessary to get rid of the superfluous male fluid, for it would soon decay and pollute the water if it were allowed to remain, and if it is not drawn off from the eggs while they are at the bottom, it is almost impossible to remove it after the embryos have begun to swim, without losing them as well.

After a final stirring, the beaker should be allowed to stand for about five minutes, to allow the eggs to settle to the bottom, and the fluid above them should then be drawn off

through a siphon, reaching nearly but not quite down to the eggs. A fresh supply of sea water should then be added, and the eggs stirred and allowed to settle, and the water drawn off as before, and this should be repeated until the water, after the eggs have settled to the bottom, remains clear.

The beaker may now be set aside where it will not be exposed to sudden changes of temperature, and the eggs will require no further attention until the embryos begin to swim, which will be in from two to six hours, according to the temperature. The little oysters must of course be supplied with fresh sea water from time to time during their development, and as they are so small that the water cannot be drawn off after they begin to swim, they must be supplied with fresh water by transferring them from time to time to larger and larger beakers. In two hours or so after the eggs are fertilized the embryos begin to swim, and crowd to the surface of the water in great numbers, and form a thin stratum close to the surface. This layer of embryos may be carefully siphoned off into a very small beaker, and a little fresh sea water added. In an hour or so there will be a new layer of embryos at the surface of beaker No. 1, and these should also be siphoned into No. 2, and this should be repeated as long as the embryos continue to rise to the surface of the first beaker. Every five or six hours a little fresh sea water should be poured from a height of a foot or more into beaker No. 2, until it is filled. The contents should then be poured into a larger beaker, and sea water added four or five times a day as before. In this way the embryos may be kept alive for a week, although they have by this time got into such a large vessel that it is almost impossible to find any of them for microscopic examination.

#### THE DEVELOPMENT OF THE EGGS.

I will now attempt a brief popular account of the changes through which the fertilized egg is gradually converted into the complex body of the adult oyster.

The body of the oyster, like that of all animals, except the very simplest, is made up of organs such as the heart, digestive organs, gills and reproductive organs, and these organs are at some period in the life of the oyster made up of microscopic cells. The eggs shown in Figures 49 and 53, will answer to illustrate the character of the cells which compose the body; each of these consists of a layer of protoplasm around a central nucleus, which, in the egg, is a large, circular, transparent body known as the germinative vesicle. Each cell of the body is able to absorb food, to grow and to multiply by division, and thus to contribute to the growth of the organ of which it forms a part. The ovarian eggs are simply the cells of an organ of the body, the ovary, and they differ from the ordinary cells only in being much larger and more distinct from each other; and they have the power, when detached from the body, of growing and dividing up into cells, which shall shape themselves into a new organism like that from whose body the egg came. Most of the steps in this wonderful process may be watched under the microscope, and owing to the ease with which the eggs of the oyster may be obtained, this is a very good egg to study.

About fifteen minutes after the eggs are fertilized, they will be found to be covered with male cells, as shown in Figure 51. In about an hour the egg will be found to have changed its shape and appearance. It is now nearly spherical, as shown in Figure 1, and the germinative vesicle is no longer visible. The male cells may or may not still be visible upon the outer surface. In a short time a little transparent point makes its appearance on the surface of the egg, and increases in size, and soon forms a little projecting transparent knob—the *polar globule*—which is shown in Figure 3, and in succeeding figures.

Recent investigations tend to show that while these changes are taking place one of the male cells penetrates the protoplasm of the egg and unites with the germinative vesicle, which does not disappear, but divides into two parts, one of

which is pushed out of the egg, and becomes the polar globule, while the other remains behind and becomes the *nucleus* of the developing egg, but changes its appearance so that it is no longer conspicuous. The egg now becomes pear-shaped, with the polar globule at the broad end of the pear, and this end soon divides into two parts, so that the egg (Figure 6), is now made of one large mass and two slightly smaller ones, with the polar globule between them.

The later history of the egg shows that at this early stage the egg is not perfectly homogeneous, but that the protoplasm which is to give rise to certain organs of the body, has separated from that which is to give rise to others.

If the egg at the stage shown in Figure 6, were split in the plane of the paper, we should have what is to become one half of the body in one part and the other half in the other. The single spherule at the small end of the pear is to give rise to the cells of the digestive tract of the adult, and to those organs which are to be derived from it, while the two spherules at the small end are to form the cells of the outer wall of the body and the organs which are derived from it, such as the gills, the lips and the mantle, and they are also to give rise to the shell. The upper portion of the egg in this and succeeding figures is to become the ventral surface of the adult oyster, and the surface which is on the right side in Figure 6 is to become the anterior end of the body of the adult. The figure therefore shows the half of the egg which is to become the left half of the body. The upper portion of the egg soon divides up into smaller and smaller spherules, until at the stage shown in Figures 24, 25 and 26, we have a layer of small cells wrapped around the greater part of the surface of a single large spherule, and the series of figures shows that the latter is the spherule which is below in Figure 6. This spherule now divides up into a layer of cells, and at the same time the egg, or rather the embryo, becomes flattened from above downward, and assumes the shape of a flat oval disk. Figures 29 and 30, are views of the upper and lower surface of the embryo at about this time.



In a sectional view, Figure 31, it is seen to be made up of two layers of cells; an upper layer of small transparent cells, *ec*, which are to form the outer wall of the body, and which have been formed by the division of the spherules which occupy the upper end of the egg in Figure 25, and a lower layer of much larger, more opaque cells, *g*, which are to become the walls of the stomach, and which have been formed by the division of the large spherule, *a*, of Figure 25.

This layer is seen in the section to be pushed in a little towards the upper layer, so that the lower surface of the disk-shaped embryo is not flat, but very slightly concave. This concavity is destined to grow deeper until its edges almost meet, and it is the rudimentary digestive cavity. A very short time after this stage has been reached, and usually within from two to four hours after the eggs were fertilized, the embryo undergoes a great change of shape, and assumes the form which is shown in three different views in Figures 32, 33, 34 and 35.

A circular tuft of long hairs or cilia has now made its appearance at what is thus marked as the anterior end of the body, and as soon as these hairs are formed they begin to swing backwards and forwards in such a way as to constitute a swimming organ, which rows the little animal up from the bottom to the surface of the water, where it swims around very actively by the aid of its cilia. This stage of development, Figure 32, which is of short duration, is of great importance in raising the young oysters, for it is the time when they can best be siphoned off into a separate vessel and freed from the danger of being killed by the decay of any eggs which may fail to develop. On one surface of the body at this stage, the dorsal surface, there is a well marked groove, and when a specimen is found in a proper position for examination, the opening into the digestive tract is found at the bottom of this groove. Figure 33, is a sectional view of such an embryo. It is seen to consist of a central cavity, the digestive cavity, which opens externally on the dorsal surface of the body by a small orifice, the primitive mouth, and which is surrounded at all points, except at the mouth, by a wall

which is distinct from the outer wall of the body. Around the primitive mouth these two layers are continuous with each other.

The way in which this cavity, with its wall and external opening, has been formed, will be understood by a comparison of Figure 33, with Figure 2S. The layer which is below in Figure 2S has been pushed upwards in such a way as to convert it into a long tube, and at the same time the outer layer has grown downwards and inwards around it, and has thus constricted the opening. The layer of cells which is below in Figure 2S thus becomes converted into the walls of the digestive tract, and the space which is outside and below the embryo, in Figure 28, becomes converted into an inclosed digestive cavity, which opens externally by the primitive mouth.

This stage of development, in which the embryo consists of two layers, an inner layer surrounding a cavity which opens externally by a mouth-like opening, and an outer layer, which is continuous with the inner around the margins of the opening, is of very frequent occurrence, and it has been found, with modifications, in the most widely separated groups of animals, such as the star-fish, the oyster and the frog, and some representatives of all the larger groups of animals, except the Protozoa, appear to pass during their development through a form which may be regarded as a more or less considerable modification of that presented by our oyster embryo. This stage of development is known as the *gastrula* stage.

Certain full grown animals, such as the fresh water hydra and some sponges, are little more than modified gastrulas. The body is a simple vase, with an opening at one end communicating with a digestive cavity, the wall of which is formed by a layer of cells, which is continuous around the opening with a second layer which forms the outer wall of the body. This fact, together with the fact that animals of the most widely separated groups pass through a gastrula stage of development, has lead certain naturalists to a generalization, which is known as the "gastrula theory." This theory or hypothesis is that all animals, except the Protozoa, are more or less direct

descendants of one common but very remote ancestral form, whose body consisted of a simple two-walled vase, with a central digestive cavity opening externally at one end of the body.

Hæckel, who is the originator and leading advocate of this hypothesis, has proposed to call this ancestral form a "Gastrea;" and the gastrula stage of development he regards as a trace or indication of this distant ancestry, which is still retained and passed through during the early stages of the development of animals which are now very widely separated.

The gastrula theory cannot be regarded as one of the established generalizations of science, and the evidence which has so far been accumulated by embryologists is not by any means straightforward or satisfactory. The theory is one of the most interesting embryological problems under discussion, however, and any new information which bears upon it is of value.

The fact that the oyster goes through a very well marked and very slightly modified gastrula stage is therefore of great theoretical interest, and more so since Salensky, a distinguished Russian embryologist, has proposed in place of the gastrula theory another theory, which is based, in part, upon erroneous observations upon the development of the oyster, which Salensky says does not pass through the gastrula stage of development at all, but forms a digestive cavity in another way.

The edges of the primitive mouth of the oyster continue to approach each other, and finally meet and unite, thus closing up the opening, as shown in Figure 36, and leaving the digestive tract without any communication with the outside of the body, and entirely surrounded by the outer layer. The embryo shown in Figures 32 and 36 are represented with the dorsal surface below, in order to facilitate comparison with the adult, but in Figure 37, and most of the following figures, the dorsal surface is uppermost, for more ready comparison with the adult. The furrow in which the primitive mouth was placed still persists, and soon a small irregular plate makes its appearance at each end of it. These

little plates are the two valves of the shell, and in the oyster they are separated from each other from the first, and make their appearance independently.

Soon after they make their appearance, the embryos cease to crowd to the surface of the water, and sink to various depths, although they continue to swim actively in all directions, and may still be found occasionally, close to the surface. The region of the body which carries the cilia now becomes sharply defined, as a circular projecting pad, the *velum*, and this is present and is the organ of locomotion at a much later stage of development. It is shown at the right side of the figure in Figure 37, and in Figure 45 it is seen in surface view, drawn in between the shells, and with its cilia folded down and at rest, as they are seen when the little oyster lies upon the bottom.

The two shells grow rapidly, and soon become quite regular in outline, as shown in Figures 37 and 44, but for some time they are much smaller than the body, which projects from between their edges around their whole circumference, except along a short area, the area of the hinge, upon the dorsal surface, where the two valves are in contact.

The two shells continue to grow at their edges, and soon become large enough to cover up and project a little beyond the surface of the body, as shown in Figure 44, and at the same time muscular fibres make their appearance and are so arranged that they can draw the edge of the body and the velum in between the edges of the shell, in the manner shown in Figure 45. In this way that surface of the body which lines the shell becomes converted into the two lobes of the mantle, and between them a mantle cavity is formed, into which the velum can be drawn when the animal is at rest. While these changes have been going on over the outer surface of the body, other important internal modifications have taken place. We left the digestive tract at the stage shown in Figure 36, without any communication with the exterior.

Soon the outer wall of the body becomes pushed inward, to form the true mouth, at a point (Figure 37), which is

upon the ventral surface, and almost directly opposite the point where the primitive mouth was situated at an earlier stage. The digestive cavity now becomes greatly enlarged, and cilia make their appearance upon its walls, the mouth becomes connected with the chamber which is thus formed, and which becomes the stomach, and minute particles of food are drawn in by the cilia, and can now be seen inside the stomach, where the vibration of the cilia keep them in constant motion. Up to this time the animal has developed without growing, and at the stage shown in Figure 36 it is scarcely larger than the unfertilized egg, but it now begins to increase in size. The stages shown in Figures 44 and 45 agree pretty closely with the figures which European embryologists give of the oyster embryo at the time when it escapes from the mantle chamber of its parent. The American oyster reaches this stage in from twenty-four hours to six days after the egg is fertilized; the rate of development being determined mainly by the temperature of the water.

Soon after the mantle has become connected with the stomach, this becomes united to the body wall at another point a little behind the mantle, and a second opening, the *anus*, is formed. The tract which connects the anus with the stomach lengthens and forms the intestine, and, soon after, the sides of the stomach become folded off to form the two halves of the liver, as shown in Figure 44.

Various muscular fibres now make their appearance within the body, and the animal assumes the form shown in Figures 44 and 45.

All my attempts to get later stages than these failed, through my inability to find any way to change the water without losing the young oyster, and I am therefore unable to describe the manner in which the swimming embryo becomes converted into the adult, but I hope that this gap will be filled, either by future observations of my own or by those of some other embryologist.

In my attempt to raise the oyster embryo from the egg, I found that continuous warm weather was essential to success.

As my observations upon the developing eggs occupied all my time, I was not able to make any record of the temperature of the water of the ocean, but during June there were a number of cold, windy days and nights, and two hail-storms, and on each of the cold days all the embryo which I had in the house died.

Before I close this portion of my paper, I wish to call attention to some points of general interest, which have suggested themselves to me during the prosecution of my work.

At first sight it does not seem possible that an animal which is encased in a hard, strong, protecting shell, and which is capable of giving rise to several million eggs every season, can be in any danger of extermination; and it seems as if the oyster ought to be able to hold its own in the struggle for existence, and to increase and multiply in the face of the most adverse circumstances.

It appears wonderful that the waters of the Chesapeake Bay are not paved with oysters, and persons who have not given much thought to the subject will ridicule the statement that there is any need for measures to prevent their extermination or the destruction of the natural beds. While the consumption of oysters was restricted to regions in the immediate vicinity of the beds, the number of oysters which it would pay to gather and put into the market each season from each bed was limited; but with the present facilities for packing and transporting oysters, there is no limit to the number which can be utilized, and the danger of destroying the best beds grows greater every day, and keeps pace with the increasing population and improvements in transportation.

Those who believe that the abundance of the supply up to the present time is sufficient proof that it will continue, will do well to reflect upon the facts given in the following table, which I have condensed from a recent book on the oyster, by Möbius (*Die Austern und die Austernwirtschaft*, Möbius, Berlin, 1877, page 67.) He gives a long table, showing the number of oysters taken yearly from the Bay of Cancale, on the coast of Norway, for about one hundred years, and I have copied enough from it to show its character:

In the year 1800 the number of oysters taken was....	1,200,000
" " " 1820 " " " " " "	.... 6,000,000
" " " 1825 " " " " " "	.... 20,000,000
" " " 1830 " " " " " "	.... 30,000,000
" " " 1835 " " " " " "	.... 43,000,000
" " " 1840 " " " " " "	.... 52,000,000
" " " 1845 " " " " " "	.... 67,000,000
" " " 1847 " " " " " "	.... 71,000,000
" " " 1850 " " " " " "	.... 50,000,000
" " " 1855 " " " " " "	.... 20,000,000
" " " 1860 " " " " " "	.... 8,000,000
" " " 1865 " " " " " "	.... 1,100,000
" " " 1868 " " " " " "	.... 1,079,000

Previous to the year 1800, and from this date to 1825, the number taken each year was small, and did not average more than five or six million oysters, and the enormous numbers which were taken from the beds in late years show that the removal of this moderate number yearly had no tendency to destroy the beds. It seems quite evident from the figures that the bed might have yielded twenty million oysters a year for an indefinite period, and the figures given for the years after 1825 are therefore highly instructive, for they show that a bed which is capable of furnishing a very great supply of oysters may be completely exterminated within a comparatively few years by unlimited dredging.

The table also shows that it will not answer to rely upon the very great number of eggs, and therefore trust to a few oysters the work of replenishing the bed.

In view of such facts, no one who appreciates the magnitude of the oyster industry of the Chesapeake can doubt that the protection of the natural oyster beds is a matter which is worthy of the most careful attention. While the manner in which this is to be accomplished is outside the scope of the present paper, a statement of those favorable and unfavorable influences which have suggested themselves to me during my work, may fairly find a place here.

It is well known to naturalists that the number of individuals which reach maturity in any species of animals or plants does not depend upon the number which are born. The

common tape-worm lays hundreds of millions of eggs in a very short time, yet it is comparatively rare. The number of children born to each pair of human beings during their lifetime of from fifty to eighty years, can be counted on the fingers, yet man is the most abundant of the larger mammals, and human population increases quite rapidly under favorable circumstances. This comparison shows plainly that the abundance of a species is determined, mainly, by the external conditions to which it is exposed, and that the number of individuals which are born has very little to do with it. In the case of the oyster, the adult is well protected against enemies by the shell, and as its food is abundant, and is brought to it by the water, it is tolerably sure of a long life after it has reached its adult form, but the life of the young is very precarious; that of the young American oyster peculiarly so, since it is exposed to all kinds of enemies and accidents, at a time when it is most helpless. The protection of the young European oyster by the parent shell at this time would seem to more than balance the greater number of eggs laid by the American.

The most critical time in the life of the American oyster is undoubtedly the time when the egg is discharged into the water to be fertilized, for the chance that each egg which floats out into the ocean to shift for itself will immediately meet with a male cell is very slight, and it is essential that the egg should be fertilized very quickly, for the unfertilized egg is destroyed by the sea water in a very short time. The next period of great danger is the short time during which the embryos swarm to the surface of the water. They are so perfectly defenceless, and so crowded together close to the surface, that a small fish, swimming along with open mouth, might easily swallow in a few mouthfulls a number equal to the human population of Baltimore. They are also exposed to sudden changes of temperature, and as my experiments have shown that a sudden fall in temperature is fatal to them at this time, the number which are destroyed by cold rains and winds must be very great indeed.

As soon as they are safely past this stage, and scatter and



swim at various depths, their danger from accidents and enemies is greatly diminished, and their chance of reaching maturity increases hundreds, and probably thousands of times.

My experiments show that there is no difficulty in developing them up to this point in the house in small aquaria, and in carrying them safely past the most precarious part of their lives, and freeing them from all their greatest dangers.

Although the mortality at these early stages is so excessive, the number of young which pass through them safely without help is very great, and if there were no other dangers and uncertainties there would be no need of measures for their protection. As they swim to and fro in the water, they are carried to great distances by the tides and currents and reach all parts of the region of water in which the parent bed is situated. In a favorable year a floating plank or bush, or piece of drift-wood, will be found to become covered with small oysters which have fastened to it, although it may not be within miles of any natural oyster bank. The fact that the young may be collected in this way in any part of the Chesapeake Bay shows that the young oysters must settle down upon the bottom in nearly all parts of the bay, and we should expect the adults to have an equally general distribution. This is far from the case, and nothing could be farther from the truth than the idea that the bottom of the waters of the oyster regions is uniformly covered with oysters, and that it is only necessary to throw a dredge overboard and drag it along the bottom for a short distance, in order to bring it up full. Nothing could be a greater mistake, for both in this country and in Europe, the oysters are restricted to particular spots, "beds" or "banks," which are as well defined and almost as sharply limited as the tracts of wood-land in a farming country. These beds are so well marked that they can be laid down on a chart or staked out with buoys; and even in the best oyster regions they occupy such an inconsiderable part of the bottom that any one ignorant of their position would have very little chance of finding oysters by promiscuous dredging. Although the young are distributed every year, by the tides and currents, to all parts of the bottom, the dredge very seldom brings up even a single oyster outside the limits of the beds.

The restriction of the oysters to certain points does not appear to depend upon the supply of food, or upon the character of the water, but almost entirely upon the nature of the bottom. The full-grown oyster is able to live and flourish in soft mud, as long as it is not buried too deeply for the open edge of the shell to reach above the mud, and draw a constant supply of water and food onto the gills. The placing of adult oysters upon such bottoms at convenient points, to "fatten" for the market, is a well-known practice. The oyster embryo would be engulfed and smothered at once if it should settle down upon such a bottom, and in order to have the least chance of survival and long life the young oyster must find some solid substance to fasten itself to, in order to preserve it from sinking in the soft mud or from being covered by shifting sand or gravel. As soon as the young oyster finds such a solid body, rough and clean, it fastens one valve of its shell to it by secreting a cement of shelly matter around the growing edge.

The living and dead shells of the adult oysters furnish the best surfaces for the attachment of the young, and for this reason the points where oyster beds are already established are those where the young have the most favorable surroundings and the best chance for life, and the beds thus tend to remain permanent and of substantially the same size and shape.

At the time of attachment the shell of the young oyster is still very thin and delicate, and the animal falls an easy victim to the numerous enemies which abound upon the oyster beds, such as crabs of various sorts, carnivorous gastropods and various fishes.

It is not an uncommon thing for fifty or a hundred young "spat" to attach themselves to one full-grown shell. Some of these are killed by enemies, and others are crowded out, so that only a few grow up at the expense of the others, and the number which survive is astonishingly small. Möbins has made an attempt to ascertain what chance of survival the newly-hatched oyster has. The ratio between half-grown and adult oysters in the oyster beds of Schleswig-Holstein has

been ascertained and recorded, at intervals of a few years, for more than a century, by a systematic method of counting the contents of a certain number of sample dredgings. From a Danish book on the subject (*Der Danske Osterbanker*, by H. Kroyer, Kjöbenhavn, 1839), and from other sources, he obtains the following series of ratios:

In 1730 there were 486 half-grown oysters to every 1,000 full-grown ones.									
" 1734	"	"	810	"	"	"	1,000	"	"
" 1740	"	"	418	"	"	"	1,000	"	"
" 1756	"	"	490	"	"	"	1,000	"	"
" 1775	"	"	484	"	"	"	1,000	"	"
" 1799	"	"	807	"	"	"	1,000	"	"
" 1819	"	"	888	"	"	"	1,000	"	"
" 1830	"	"	417	"	"	"	1,000	"	"
" 1839	"	"	440	"	"	"	1,000	"	"
" 1852	"	"	473	"	"	"	1,000	"	"

or an average of 421.2 to 1,000, or 42.13 per cent. of young. The uniformity of the ratio between the half-grown and the adult oysters, through a period of more than one hundred years, is very remarkable. In no case was there less than 30 per cent. of young or more than 50 per cent., and the average of 42 per cent. is very closely followed. After an oyster has become half-grown its dangers are very few, and this number—42—probably gives with sufficient accuracy the number of oysters which grow up each year for each 100 adults. Although the number of young which are born each year is great beyond computation, those which survive are less than half as numerous as the adults.

Möbius has estimated the number of adults which spawn each year, and multiplying this number by the average number of eggs laid by each, and dividing by the number which grow up, he reaches the conclusion that each oyster which is born has  $\frac{1}{1,145,000}$  of a chance of reaching maturity. In the case of the American oyster, the number of eggs is very much greater, and each one's chance of survival is accordingly very much less, and it is evident that the great fertility of the oyster will not protect a bed from destruction by excessive dredging, for while the young spat frequently cover the

shells of the adults by hundreds, the number of half-grown oysters is always less than one-half the number of adults. The great mortality of the young, after they have fastened themselves to the shells of the adults, is due in part to want of room, in part to the attacks of enemies, in part to accidents, such as the shifting of the bottom, and in part, no doubt, to lack of food. While the supply of organic matter which is carried to them by the water is very great, it is not unlimited, and the amount which each oyster can obtain at any one time is quite small, and if the oysters covered the bottom in sufficient abundance, some of them might fail to obtain a sufficient supply. I do not believe, however, that this ever occurs, for long before the oysters are sufficiently abundant to exhaust the supply of organic matter, their numbers are limited by other conditions. The growth of an animal does not depend upon the supply of food in general, but upon the supply of the least abundant of the necessary ingredients of the food. It is well known that a field that is very fertile will fail to produce a satisfactory crop of a plant which needs some particular food-ingredient which the soil contains in too small quantity. Although food in general is very abundant, the growth of this particular crop depends upon the amount of this ingredient, and while the seed which has been planted yields an abundant crop of young plants, only a few are able to grow up, and those can grow no faster than they can extract this particular ingredient from the soil.

In addition to organic food, the oyster needs a supply of carbonate of lime to make its shell, and this is supplied to it, in solution, in sea-water. If the shell is thin, or if it is formed very slowly, the danger from enemies and accidents is greatly increased; and those oysters which are able to construct their shells with the greatest rapidity are the ones which survive and grow up. The amount of dissolved carbonate of lime which the ocean contains is unlimited, but the amount which can reach each oyster is not very great; and if all the oysters which attach themselves were to survive there can be no doubt that they would exhaust the available supply of lime before they failed to obtain enough organic food.

It is well known that shell-fish of all kinds thrive best when the supply of lime is greatest. The fresh-water mussels which live in streams and ponds where the supply of lime is scanty, grow slowly, and their shells are so thin that they are very subject to accidents, and their numbers are limited; but in limestone regions the shells are large and heavy, and the bottoms of the streams are almost paved with mussels, and it is well known to conchologists that coral reefs and islands are the most favorable regions for the abundant growth of all kinds of shelled molluscs.

The dead oyster-shell is soon corroded, and in a few years entirely dissolved, by the sea-water; and I think this fact is another reason why the young oysters thrive best on a natural oyster-bed.

How far the supply of oysters is limited by the supply of lime it is impossible to say; but when we recollect how important it is that the young oysters should soon find solid bodies to fasten themselves to, and that they should protect themselves by strong shells of their own as quickly as possible, it will be seen that the danger of exterminating a natural bed by over-dredging would be much less if the empty shells were replaced upon the bed. There can be no doubt that the best natural beds may be destroyed by over-dredging, and that this fate will be certain to overtake the beds of the Chesapeake Bay if the oyster industry continues to increase, and matters are left to adjust themselves.

Like most dangers, this is one which will not become conspicuous until it is too late to find a simple remedy.

Whether the number of oysters which are at present taken from our oyster beds is too great or not is a matter which is outside the field which I undertook to investigate, but it is a matter in which the public have the greatest interest, and the extent of the oyster beds, and the number of oysters which they can supply each year, should be accurately ascertained before it is too late.

A prophet of future evil is always regarded as an unseasonable croaker, but the facts which I have noticed seem to show that, whether the danger of exterminating the best and most

accessible oyster beds is near or remote, it is sure to force itself upon us some time. If we wait for that time the remedy will be slow and expensive, but prevention of the danger in time would not necessarily be attended with any very great expense.

The investigation into the hydrography and general condition of the oyster beds of Tangier and Pokamoke Sounds, which has been so ably conducted by Captain Winslow, of the United States Coast Survey, during the past two summers, will supply the necessary information; and should these investigations show that any of these beds are in immediate need of artificial help to save them from destruction, I hope that the observations I have detailed in this paper may point to the way in which this help may be given.

## PART II—EMBRYOLOGY.

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### THE SEXES OF THE OYSTER.

A list of the contradictory opinions which various writers have expressed regarding the distinctness of the sexes of the oyster is hardly worth publishing, since all the thoroughly competent observers who have investigated the subject in modern times agree that each oyster is, at the breeding season, either a male or a female.

During my investigations I submitted more than a thousand oysters to microscopic examination. My studies were carried on during the breeding season, and I did not find a single hermaphrodite. The male cells are so small compared with the eggs that it would be impossible to state that a mass of eggs taken from the ovary contained no spermatozoa, although they could not escape detection if they were at all abundant.

On the other hand, a single egg in the field of the microscope in a drop of male fluid would be very conspicuous, and could not escape detection; and the fact that not a single case of this kind occurred is sufficient to establish the distinctness of the sexes at the breeding season.

A short time since, October 25th, I examined a number of oysters with the same result. I found six females with ovaries filled with nearly ripe ovarian eggs, and eight males whose testes were filled with spermatozoa, most of which were immature, but some of them fully developed and active.

One of the males and one female were hardened in chromic acid, and a number of microscopic sections were cut from various parts of the reproductive organs of each. None of the ovarian follicles or parts of follicles of the female contained anything but eggs, and not a single egg at any stage of development was found in any part of the body of the male.

In order to show how conspicuously the characteristics of the sexes are shown in these sections I have made careful drawings of two of them. Figure 53 is a section of part of the visceral mass of the female, showing nine ovarian follicles cut in various directions. Each follicle contains a nearly central cavity, and around this, on all sides, the opaque granular eggs project from the basement membrane of the follicle, to which they are attached either directly or by long stalks. Each egg contains a large, conspicuous, oval, transparent nucleus, and a single nucleolus, which is on that side of the nucleus which is nearest the point of attachment to the membrane. The eggs are so crowded that they are flattened and rendered polygonal by mutual pressure, and in some places there is a very regular alternation of those with and those without stalks.

Figure 67 is a section of a portion of the visceral mass of a male, as seen with the same magnifying power. The space nearest the basement membrane of each follicle is here occupied by a thick layer of small cells, the mother cells of the spermatozoa, and the centre of the follicle, instead of being empty, as in the female, is filled with a mass of spermatozoa, which have been set free. Some of the follicles shown in this section open by slightly constricted orifices into a large oval duct, with a lining of epithelial cells. These ducts are filled with nearly ripe spermatozoa, which have been forced into them from the follicles, and even in the hardened specimen traces of the movement of the spermatozoa from the cavities of the follicles into that of the duct are retained. A comparison of this figure with the one just described shows the male follicle to be so different from the female follicle in structure and appearance that the occurrence of a male follicle in a section of the ovary, or of a female follicle in a section of the testis, could not escape instant detection, and it is also plain that one of the very characteristic ovarian eggs shown in Figure 53, could hardly fail to attract attention at once if it should occur in a section of the testis.

My observations tend to show, then, that the sexes are separate in the American oyster of the Chesapeake Bay during



the summer months and up to the end of October, but the present season has been unusually mild, and Capt. Winslow, U. S. N., tells me that he found oysters in October of this year in Tangier Sound, the eggs from which he succeeded in fertilizing. It is possible, then, that those which I examined were ready to spawn this season. If this is so, all my observations have been made during the breeding season, and it may be possible that later in the year hermaphrodite individuals may be found. There is nothing improbable in the statement that oysters change their sex, and that an individual may be a female one year and a male another year, but my observations certainly do not indicate that this is the case with the American species.

The only observations which I have met with on this subject, made in this country, are by McCrady (Observations on the Food and Reproductive Organs of *Ostrea Virginiana*, with some Account of *Bucephalus Cuculus*: Proceedings Boston Soc. Nat. Hist., December 3d, 1873, pp. 170-192). He says that at a time when the ovarian eggs are very small and immature "the spermatozoa may be seen in their aggregated or even their free condition, actively moving about among masses of granular yolk substance, inclosing many germinative vesicles, without exhibiting any attraction for them, and without the appearance of any change in the young vesicles themselves." (Page 172.)

Regarding the oysters nearer the breeding season, when the eggs were more mature, he says, p. 174: "I endeavored next to ascertain whether or not spermatozoa were present, but could not satisfy myself on this point, as my eye had become fatigued, and no disposition of the light enabled me to discover whether the minute dancing cellules, which were quite numerous, had or had not a tail."

This observation would seem to indicate that, while the sexual elements are immature, both male and female elements may occur in the same individual.

The most thorough and satisfactory observations upon the sex of the European oyster are those by Möbius, and his account shows that the sexes are separate at the breeding

season. His observations were published in 1871, under the title "Untersuchungen über die Fortpflanzungsverhältnisse der Schleswigischen Austern. Nachr. der deutschen Malak. Gesellsch. III, 1871. Abstr. in Hoffmann u. Schwallbe's Jahresberichte, 2, 1873, p. 338. In his most recent publication on the oyster (Der Auster und die Austern-wirtschaft, Berlin, 1877), he reviews the subject and says, p. 19: "Die Austern sind Zwitter. In einer grösseren Zahl Austern fand ich in der ganzen Geschlechtsdrüse nur Befruchtungskörper, aber keine eier. Bei 7 Austern, die blau Brut im Barte trugen, enthielt die Geschlechtsdrüse Befruchtungskörper."

It would seem to be at least as probable that these seven oysters were males which had drawn floating embryos in onto their gills as that they were females which had changed to males after laying all their eggs.

"Drei Austern mit jüngeren weissen Keimen im Barte hatten keine Befruchtungskörper in der Geschlechtsdrüse. Bei den meisten Keimenträchtigen Austern enthielten der Geschlechtsdrüse weder Eier noch Befruchtungskörper. Von 309 Austern, welche am 25 Mai auf vier verschiedenen Bänken im Osten der Insel Sylt gefischt und von 26 Mai bis 1 Juni untersucht wurden, waren 18 Procent geschlechtlich unentschieden, der übrigen 82 Procent waren zur Hälfte eierträchtig, zur Hälfte spermaträchtig. Bei keiner waren die Geschlechtsproducte ausgereift. Aus diesen Befunden schliesse ich dass in der Geschlechtsdrüse der Austern nicht gleichzeitig, sondern folgezeitig Eier und Befruchtungskörper entstehen; dass Befruchtungskörper sehr bald nach dem Ausstossen der Eier entstehen können, und dass wahrscheinlich der eine Hälfte der Austern einer Gebietes in einer Brutperiod bloss Eier die andere Hälfte bloss Sperma bildet."

His conclusion that the sex of the oyster changes after the reproductive elements have been discharged from the body, is thus seen to rest upon the occurrence of spermatozoa in the reproductive organs of oysters whose gills carried embryos, but as it seems perfectly possible that these might have escaped from the mantle chambers of the oysters, and thus gained access to the gills, there is no proof that these oysters had ever acted as females.

Lacaze-Duthier's observations, published more than twenty years ago (*Ann. d. Sc. Nat.* 1854. *Organes génitaux des Acéphales Lamellibranches*; and, *Comptes rendus*, 1855, x 4, 415-420. *Des organes de la generation de l'huitre*), are very similar to those of Möbius. He says that at any given time each oyster is almost exclusively male or almost exclusively female, and he thinks that the young oysters are functionally male, and become female as they grow older.

As I have already stated, I have found oysters only one year old which contained ripe eggs, and eggs only, and others of the same age which were exclusively male, and I have succeeded in fertilizing the eggs of the one with the fluid of the other. This observation, which is corroborated by Gerbe's statement (*Zool. Record*, 1876, xiii., *Mol.* p. 62), that among 435 European oysters one year old, he found 35 with young; 127 with ripe eggs, and 189 with ripe semen, seems to be sufficient to show the incorrectness of Lacaze-Duthier's conjecture that the functionally male condition precedes the functionally female condition.

#### MANNER OF FERTILIZATION.

Although the American oyster seems well adapted, like the European species, and various other marine and fresh-water Lamellibranchs, to draw into its mantle-chamber, with the seawater, the spermatozoa discharged from the mantle-chambers of neighboring oysters, and thus to bring about the fertilization of the eggs inside the cavity of the shell, this does not seem to occur.

I have carefully searched the gills and mantles of more than a thousand oysters at a time when the reproductive organs were plainly seen to be discharging their ripe contents, and have not found a single fertilized egg or embryo in any part of the mantle-chamber, in or on the gills, or anywhere else inside the shell. This negative evidence, together with the fact that the eggs can be hatched after they have been artificially removed from the ovaries seems sufficient to prove, in the absence of all evidence to the contrary, that the eggs of the American oyster undergo development in the open ocean.

The various observations which have been published regarding the place where the eggs are fertilized in the European oyster are very contradictory.

In 1827 Home stated (Phil. Trans. 1827,) that the eggs are impregnated inside the ovaries; but as his paper also states that the rotation of the ciliated embryo is caused by a parasitic worm, it is doubtful whether the means at his command were adequate to the solution of the question.

In the "Report of the Commission appointed to Examine into the Methods of Oyster Culture in France and in the United Kingdom with a view to the Introduction of Improved Methods of Culture of the Oyster into Ireland, 1870," J. G. Hart, Esq., one of the Commissioners, says, p. 10: "Artificial fecundation, such as is practiced with the Salmonidae, is impossible, from the fact that fecundation takes place before the extrusion of the ova from the ovaries, and therefore we must conclude that with the oyster the utmost that can be done by so-called artificial breeding is not the procuring of artificial impregnation, but only the shepherding of the impregnated ova during infancy." The five original figures which he gives, pp. 9 and 11, Figs. 1, 2, 3, 4 and 5, as well as his account of the early stages of the oyster, are so crude and indefinite as to throw great doubt on the value of his evidence.

In a work entitled "Guide Pratique de l'Ostreiculture," Prof. Felix Fraiche makes a similar statement, that since the eggs are fertilized within the ovaries artificial fertilization is impossible, but his statement does not appear to be based upon observation.

Eyton, who appears to be a thoroughly competent observer, states (History of the Oyster and Oyster Fisheries, by T. C. Eyton, F. L. S., F. Z. S.: London, 1858, p. 21) that in a number of oysters which he has opened and examined at various times, and from different places, embryos, at different stages of development, were present inside the ovaries as well as on the gills. There seems to be no reason for doubting his evidence, but it does not seem to be sufficient to show that the eggs are fertilized exclusively in the ovaries.

Möbius, whose statements rest on careful observation, states

(Die Austern und die Austern-wirtschaft, p. 17) that the oysters discharge their ripe eggs into the gills, and that they commence their development after they have left the reproductive organs

The occurrence of unfertilized eggs on the gills is conclusive evidence that impregnation does not always take place in the ovaries, but it cannot be regarded as evidence that the eggs have not been discharged from the cloaca into the external water, and then drawn back into the mantle chamber. It seems possible that some, at least, of the eggs of the European oyster may be fertilized outside the shell, as is the case with the American species; but there does not at present seem to be any reason to believe that they ever complete their development elsewhere than inside the shell. It is, of course, impossible for an American to decide this point, but I think it is one to which the renewed attention of European naturalists might well be directed.

#### SEGMENTATION.

The segmentation of the oyster egg is remarkable for its great rapidity, for its bilateral symmetry, and for the very well marked alternation of periods of activity with periods of rest. The rate of segmentation varies greatly according to temperature and other conditions, but in some cases the ciliated embryo is formed within two hours after fertilization. After the completion of the first division the position of the right and left sides, as well as that of the dorsal and ventral surfaces, is determined.

The ripe, unfertilized egg is quite variable and irregular in shape, usually elongated and pear-shaped, but sometimes polyhedral, and without the stalk, round over part of its surface and flattened over part, or even perfectly spherical. The characteristic shapes of the eggs are well shown in 49, 51 and 53. No external membrane is visible in the unfertilized egg, and, as shown in the figures, the protoplasm of the yolk forms a thin, slightly granular layer around the very large oval, transparent germinative vesicle, which again contains a single, more highly refractive, germinative dot. In

from five minutes to an hour after impregnation the egg becomes quite regularly spherical, as shown in Figure 1, and is now covered by a distinct limiting membrane, which adheres closely to the surface of the uniformly granular yolk.

The changes of segmentation take place so very rapidly that the close observation of the living egg demanded all my attention, and I was not able to make any observations upon stained specimens, regarding the fate of the germinative vesicle, or the origin of the polar globules or formation of the first segmentation nucleus; and the living egg is sufficiently opaque to prevent observations upon this point.

After the egg has assumed the spherical form shown in Figure 1, it remains without change for some time, usually nearly an hour, and then enters upon a period of activity, during which changes follow each other with great rapidity.

The first thirteen figures were drawn from the same egg. As the day was very cold, the changes were slow, and the first period of activity did not set in until two hours and seven minutes after impregnation, but the series of changes shown in Figures 2-13 occupied only seventeen minutes, and this was so much longer than usual that I was able to commence my series of drawings by sketching them.

As shown in Figure 2, the egg commences its activity by elongating and becoming oval, with one end narrower than the other. The narrow end is to become the nutritive pole, and the broad end the formative pole of the segmenting egg. In all the figures of segmenting eggs the formative pole is above, and the nutritive below, and the latter corresponds, in a general way, to the dorsal surface of the embryo.

Contractions now begin to make their appearance at the formative end, throwing the limiting membrane into waves or wrinkles, which travel rapidly towards the nutritive pole, near which they disappear.

The wrinkles are shown in Figure 2. It is, of course, impossible to show their movement in a drawing, but the progression over the surface of the yolk, from the starting-point at the small end to the place where they disappear near the round end, is well marked, and is a constant characteristic,

which may be found in every egg by patient watching. It is of very short duration, and the limiting membrane usually becomes smooth again in about fifteen seconds after the contractions commence. Before I discovered that similar waves run over the surface of the egg for a few seconds at the beginning of the active changes at later stages of segmentation, I naturally inferred that they were connected with the extrusion of the polar globules. While it seems probable that they are in some way connected with this extrusion, their occurrence at later stages shows that this is not their only significance.

So far as I am aware, this is the first notice of their occurrence.

Soon after the waves commence, an area, which is a little less granular than the mass of the egg, becomes visible at the formative pole, and from this the first polar globule soon begins to protrude, pushing out the external egg-membrane. Figure 3 is the same egg two minutes later, and Figure 4 is the same after another interval of two minutes. The oval outline is now gradually changing to a pear shape, the stalk of the pear occupying the nutritive pole, and the polar globule projecting from the middle of the broad end of the pear at the formative pole. During these stages the granular matter of the yolk may be seen to flow in a steady, slow current, around the periphery of the egg, but, as far as I could observe, the current has no definite starting-point or terminus. At these stages there are no waves on the surface, and the membrane is smooth. It is interesting to observe that while these changes are taking place the nutritive end of the egg grows a little more transparent than the formative end, a reversal of what occurs in almost all other eggs which pass through unequal or irregular segmentation, although Lovén has described the same phenomenon in *Crenella*. In a short time three planes of cleavage run in towards the centre of the egg from three equidistant points on the periphery, as shown in Figure 5, which is two minutes later than Figure 4, although the changes usually take place much more rapidly.

One of these planes is at the point occupied by the polar globule, and the others about midway between it and the

nutritive pole, and two of the three nearly equal masses into which the egg is blocked out lie at the formative end, and one at the nutritive end; the latter is less granular than the pear. The three furrows do not tend to meet exactly at the centre, but as shown in Figure 6, two minutes later than Figure 5, the one which runs down from the polar globule inclines to one side so as to meet one of the side furrows before the other. During the stages shown in this and the preceding and succeeding figures the protoplasm of the whole egg is violently disturbed, and the granular matter quivers or dances, with what would be called "Brownian" motion, were it not confined to this particular stage of development. At the stage shown in Figure 5, the three lobes *a*, *b* and *c* of the trefoil are nearly equal in size, but at the present stage, Figure 6, the one at the nutritive end, *a*, is the largest, and the one which is most perfectly separated, *c*, the smallest. The later stages show that this smallest spherule is posterior to the larger one, and this figure therefore gives a view of the left side of the egg or embryo with its dorsal surface below. For the sake of brevity, we may now call the smallest spherule, *c*, of Figure 6, the first micromere, the next largest, *b*, the second micromere, and the one at the nutritive pole, *a*, the macromere.

In one minute after the stage shown in Figure 6 the vertical furrow has united with one of the lateral furrows, to separate off the first micromere, and the second lateral furrow has run in and united with the line thus formed, so that the egg is now divided into three separate masses, each of which now becomes spherical, as shown in Figure 7. This stage ends the first period of activity, and the changes which follow result in the gradual obliteration of the sharply defined characteristics which have been acquired.

In forty-five seconds after the stage shown in Figure 7 the two micromeres, *b* and *c*, have approached and united with each other, as shown in Figure 8, and the second micromere, *b*, has also become fused with the macromere, *a*, although the egg still has its trefoil outline, and is now very similar to the stage shown in Figure 5. In another minute Figure 9, the fusion of the second micromere, *b*, with the macromere, *a*, is



much more marked, and the first micromere *c* has also begun to unite with the macromere. Up to this time the lines of union of the three spherules have been visible, but in another minute, Figure 10, there is no line to indicate the fusion of the second micromere *b* with the macromere *a*, and the primitive distinctness of the two is only indicated by a depression in the outline, which soon disappears entirely, as is shown in Figures 11 and 12. At the same time the first micromere, *c*, becomes more completely united to the mass, *a* and *b*, formed by the fusion of the second with the macromere, although, as shown in Figures 11, 12, 13, 14 and 15, the line of separation never becomes invisible. Of these, Figure 11 is three minutes later than Figure 10, and Figure 12 two and three-quarters minutes later than that. The next figure, 13, is from another egg, but is about two minutes later than 11, and shows the characteristics at the end of the second period of rest. At some time during the stages of which I have been speaking the egg sheds an external membrane, and I have copied this drawing here in order to show the membrane, nearly cast off. It will be seen that the casting of this membrane does not leave the surface of the yolk exposed, but that it is still covered with an investing membrane. I am unable to say whether the membrane was originally more than one layer thick, or whether a new one is formed to replace the one which is shed. The time when the egg escapes from this membrane varies greatly, but it is usually earlier than this stage, and an egg at the stage 10, in the act of escaping, is shown in Figure 46. At the stage 13 the egg is again almost spherical, and consists of two masses, a large one, *a* and *b*, and a small one, *c*, meeting each other upon a flat surface. The preceding stages show that the larger mass is compound, and made up of the macromere and second micromere, but there is no visible indication of this fact. The long axis of the egg, at this stage, does not pass through the polar globule, but parallel to it.

At the stage which corresponds to this the eggs of many molluscs have a segmentation cavity, but there does not seem to be any space between the two spherules of the oyster-egg,

nor does a segmentative cavity make its appearance until much later.

From the stage shown in Figure 1, up to the stage shown in Figure 10, no traces of a segmentation nucleus could be made out in the living egg, but at the stage shown in Figure 11, a large, circular, transparent body appears in the first micromere and another at the formative end of the compound mass formed by the fusion of the second micromere with the macromere.

At the end of the second period of rest (Figure 13), these bodies are much larger, and their outlines are very clear. The commencement of the second period of activity is shown in Figure 14, which is six minutes after Figure 13. The two spherules swell up and become much more conspicuous than they were at the stage before, although they are still in contact over a considerable area. The wave-like motion, noticed at an earlier stage, is now repeated in each spherule, and runs over the surface from the point furthest from the polar globule towards the end where this is situated; the wave continuing for about half a minute. At the time this motion commences a remarkable change takes place in the two transparent vesicles already mentioned. Each of these becomes irregular and star-shaped, and long channels radiate from it into the substance of the yolk, as shown in Figure 47. The central chamber then instantly disappears; the radiating channels are visible for a fraction of a second longer, and then disappear, and the places which the two large chambers had occupied are now (Figure 14), seen to be occupied by two small refractive nuclei. I at first thought that the radiating channels might be the same as the star-shaped figures of recent embryologists, but it seems more probable that each of the large chambers of Figures 11, 12 and 13, contains a nucleus which might be brought out by reagents, and which is surrounded by a more fluid substance, the diffusion of which through the yolk precedes the formation of the amphiaster and the division of the nucleus. It is possible that this diffusion is the cause of the peculiar star-like arrangement of the granules of the yolk around the nu-

cleus. The disappearance of the large chamber by a sudden contraction, and the diffusion of its contents through radiating channels, are phenomena which are as unmistakable as the somewhat similar changes of the contractile vacuole of an infusorian, although, like the latter, they are somewhat difficult to discover, and can only be seen by keeping the egg under constant observation.

In some instances, I was able to actually observe the disappearance of the germinative vesicle of the oyster egg. In many Lamellibranchs this body has considerable elasticity, and in Anodonta it may be forced by pressure through a small fissure in the ruptured egg-shell, and it will regain its original shape and size after it has escaped from the egg. This is not the case in the oyster, and in the ripe egg the vesicle seems to be almost as fluid as water, and cannot be pressed out of the yolk. Like all the changes in the oyster egg, the disappearance of the germinative vesicle takes place with great rapidity, and the manner of disappearance is identical with that which I have just described in the case of the segmentation nucleus. It becomes irregular; radiating channels run off from it into the yolk; the central chamber vanishes, and the channels are visible for an instant longer, and then disappear. The yolk is so opaque that I was not able to see that any part of the vesicle was left behind as a pronucleus, but this is probably the case.

It is useless to speculate at present upon the significance of these highly suggestive changes, but they certainly show that we may hope for very interesting results from the minute histological study of the eggs of marine Lamellibranchs.

In this connection, I may call attention to a point in the history of the "Auerbach's figures." It is not generally known that these were first figured by Carus, more than fifty years ago, in the egg of a Lamellibranch. His figures of the segmenting egg of *Unio* (*Neue Untersuchungen über die Entwicklungsgeschichte unserer Flussmuschel. Von Dr. J. G. Carus. Nova Acta, 1832, 8, 1*), show these structures about as they are represented by Flemming, but he regards them

as the plates of a calcareous shell, and compares the egg to a sea-urchin.

To return to the segmentation ; immediately after the large vesicles have disappeared, the egg has the appearance shown in Figure 14. One minute later (Figure 15), the first micromere, *c*, has become nearly spherical, and stands out sharply from the remainder of the egg, and the compound mass, *a* and *b*, of the preceding figures, is again separated into the macromere, *a*, and the second micromere, *b*. In another minute (Figure 16), the formative pole is divided into four micromeres, one of which, *b*, is only slightly marked off from the macromere, while the three others are more distinct. The impossibility of seeing both sides of the egg at once makes it difficult to say just how these four spherules are formed, but it seems most probable that two of them are formed by the division of the first micromere, and two by the division of the second.

In the egg which was figured the nearest spherule bears every indication of an origin by the division of the first micromere, *c*, into two, and other eggs served to show with equal clearness that one of them is also separated off from the second micromere, *b*. Certain irregular forms of segmentation, which will be described later, also appear to sustain this view.

Owing to an unfortunate oversight, the dotted lines which should connect the letters of reference with the parts they refer to were not copied in the drawings from which the photo-electrotypes of Plates II. and III. were made; but I hope that a more careful description will supply a remedy for the accident. Two minutes later, Figure 17, three of the micromeres, the first, *c*, and the two new ones, *d d*, are well defined and prominent; but one of them, the second micromere, *b*, has again begun to become fused with the macromere, *a*. After another interval of three minutes and a half, Figure 18, this micromere has become completely fused with the macromere, to form a compound mass, *a* and *b*, which is almost spherical, and in this a single transparent vesicle has made its appearance. The other three micromeres are even more

sharply separated than at the preceding stage, and a vesicle has made its appearance in each. After two minutes and a half, 19, these three micromeres have flattened down against each other and against the compound mass, so that the egg is once more nearly spherical. This stage may be called the third period of rest.

This figure and the one before it are from the same egg, as indeed are all the figures on this plate except 25, 27 and 28; but after Figure 18 had been sketched, the egg rotated a little, and Figure 19 is a view at right angles to all the preceding ones.

The vesicles are seen to be a little larger than they were a few minutes before, but I did not succeed in seeing them disappear at this stage.

The egg which was figured remained in the condition shown in Figure 19 for thirteen minutes, and during this time it rotated back again into the position which it had occupied at first.

At the end of thirteen minutes the three micromeres *c, d, d'*, again became conspicuous; the compound mass, *a* and *b*, elongated, and a surface depression separated the portion *b* from the portion *a*, and the first micromere, *a*, quickly divided into two, as shown in Figure 20.

By moving the cover-glass, I managed to rotate the eggs a little, and to get a sketch, Figure 21, in the same position as Figure 19. Figure 21 is one minute later than Figure 20, and it will be seen that the second micromere, *b*, and two of the others, have already begun to flatten down and to pass into the resting condition.

From this time on I was not able to keep the egg under constant observation, but examined it at short intervals. A well marked resting period follows the stage shown in Figure 21, but as it presents no new features, it was not figured. Figure 22 is fifteen minutes later, and shows the egg at the fourth period of activity. There are now two more micromeres, which appear to be formed by the two, *c c*, Figure 20, which were produced during the third period of activity by the division of the first, Figure 14, *c*.

The egg is now made up of one large micromere, *a*, at the nutritive pole, and at the formative pole six small, distinct spherules, *cc*, on one side of the polar globule, and one large one, *b*, on the other side; this is flattened, in contact over a large area with the macromere, and is, without doubt, the second micomere of earlier stages.

The history of the later stages shows that the single micromere, *b*, of this stage is anterior to the polar globule, while the group of six is posterior to it. The single macromere occupies what is to become the dorsal surface, and this figure is accordingly a view of the left side. For the sake of brevity, I shall in future use the terms dorsal and ventral, right and left, and anterior and posterior in describing the embryo, and it will be convenient to make this figure a reference mark. The ventral surface is here above, the dorsal below, the left side towards the observer, and the anterior end on the right side of the figure.

Figure 23 is a view of the posterior surface of the same egg twenty-three minutes later. The micromeres which are posterior to the polar globule have now increased in number, and form a cap—the ectoderm—resting upon the macromere. The number of spherules, or ectoderm cells which form this layer, now increases rapidly by the division of large cells into smaller ones, and two couples which have been formed in this way are shown in the figure. The ectoderm is also increased by the separation of new spherules or micromeres from the macromere at the point where this touches the posterior border of the ectoderm. This portion of the ectoderm may therefore be called the growing edge.

Figure 24 is a view of the left side of the same egg five hours and fifteen minutes later. The anterior margin of the macromere is still separated from the polar globule by a single spherule, the second micromere, *b*, but posteriorly and at the sides the layer of ectoderm has grown considerably. At five points on the exposed side there are pairs of small cells, each of which has been formed by the division of a larger one. Figure 25 is another egg in nearly the same stage of development, but it has been copied here in order to show

the separation of a new micromere, *g*, from the macromere, *a*, at the growing edge of the ectoderm.

Figure 26 is a view of the same egg as Figures 22, 23 and 24, but fifty-five minutes later than Figure 24. The ectoderm cells are now much smaller and more numerous, and the macromere is almost covered by them. At the growing edge *g*, a new micromere is separating from the macromere, and there are now a number of small cells on the median line anterior to the polar globule. In dead eggs at this stage a transparent cavity separated the inner surface of the layer of ectoderm from the macromere, but this space does not appear to be normal, and the macromere seems, in living eggs, to be in contact with the outer layer, and there is no indication of a segmentation cavity. In many respects the segmentation of the oyster egg is very similar to that of the egg of *Unio*, as described by Rabl, but in *Unio* the segmentation cavity is present at a much earlier stage than this.

From this point on I made no attempt to trace the changes of individual eggs, but made sketches of new stages as I found them. The stages which are figured here are by no means all which were observed and sketched; and I found a number of embryos intermediate between nearly all the stages which were reproduced, so that my series was much more complete than the series of figures.

Figure 27 is a surface view of the left side of an embryo twenty-seven hours after impregnation, and Figure 28 is an optical section of the same embryo. The outline of the body has undergone considerable change, and the longest axis is now the axis which runs from the polar globule to the posterior end, and the vertical axis, which was the longest during the earlier stages, is now the shortest. In a view from above or below the outline is elliptical. The optical section, Figure 28, shows that the macromere is now divided into two large spherules, *en*, which are almost entirely covered by the ectoderm, *ec*, except over a small area on the dorsal surface. The polar globule is now separated from the anterior edge of the ectoderm by four cells, which are smaller than those at the opposite or growing edge. Figure 29, is a view of the

dorsal surface or nutritive pole of a somewhat older egg, showing the two spherules of the endoderm uncovered by the ectoderm. The flattening of the embryo at the ends of the vertical axis, which had made its appearance at the stage shown in Figure 27, has now become more pronounced, and the body is nearly disk-shaped, with its dorsal and ventral surfaces flattened and parallel. The two endoderm cells now divide up, and a short time after the stage last figured they are six in number, as shown in Figure 30, which is a view of the ventral surface; the dark endoderm cells being visible through the more transparent ectoderm. Figure 31 is an optical longitudinal section of a somewhat older embryo, represented with its dorsal surface to the right, and its anterior end above. The flattening of the upper and lower surfaces is well shown in this figure. At about this stage, or a little earlier, the ectoderm and endoderm separate from each other, and a well marked segmentation cavity, or, more properly, a body cavity, is now clearly visible between them. The endoderm has now divided up into a number of large spherules, forming a layer which is pushed in towards the ectoderm, so that the dorsal surface is no longer flat, but saucer-shaped, thus forming a wide, shallow cavity, the primitive digestive cavity, *g*. On the ventral surface the ectoderm cells now carry a few short scattered cilia, and the embryo begins to swim or rotate a little.

It now undergoes considerable change of form, and in a few hours it presents, when seen in a side view, the form shown in Figure 32.

This is a surface view of the left side, and Figure 33 is an optical longitudinal section of the same embryo. This stage is of great importance in the attempt to raise the young from artificially fertilized eggs, for the velum now makes its appearance, and the embryos swim to the surface of the water, where they form a dense layer, which can be siphoned off into a supply of pure sea water, leaving the dead eggs behind. The outline at this stage is very irregular, but perfectly definite and characteristic, although the great activity of the em-



bryo renders this, in the oyster, as in most other molluscs, the most difficult stage to study.

I give three surface views of it (Figures 32, 34 and 35), in order to show the characteristics of the various aspects. Figure 32 is a view of the left side, with the anterior end to the right and the dorsal surface below. Figure 34 is an anterior view of the ventral surface, that is, a view of the upper right hand surface of Figure 32, and Figure 35 is a view of the dorsal surface. In both 34 and 35 the anterior end is below. In the embryo from which these figures were drawn the polar globule was not present, but in other embryos it occupied the centre of the tuft of cilia of the velum, as shown, at a later stage, in 36, so that there can be no doubt that the velum occupies that end of the embryo which is above in Figure 31, and at the right in most of the preceding figures.

Near the centre of the ventral surface—the top of Figure 32—there is a well marked and constant protuberance of the body wall, which occupies the region which, in most molluscan embryos, gives rise to the foot, and which may perhaps be regarded as a rudiment of that organ. In front of this protuberance the anterior end of the body is round, and is occupied by the long cilia of the velum, which form a complete closed circlet. In the centre of the dorsal surface the body is crossed by a deep crescent-shaped furrow, 32, 34 and 35 *g*, which is transverse to the long axis of the body, and which is seen in an optical section, 33 *g*, to be prolonged into the body as the primitive digestive cavity.

Posterior to this the body terminates in a pointed protuberance, 32, 33 and 35 *a*, which is of importance in determining the relation between this and later stages, and which may be called the anal papilla. A comparison of Figure 33 with Figure 31 indicates that the present form of the body has been brought about by the infolding of the edges of the disk-shaped embryo, Figure 30, towards the dorsal surface, in such a way as to carry the endoderm into the centre of the body, thus giving rise to a primitive digestive cavity, with a dorsal blastopore situated in the centre of a crescent-shaped transverse furrow. Rabl has figured a stage in the develop-

ment of *Unio* (*Entwicklungsgeschichte der Malermuschel*, Figures 28 to 32, which is very similar to this, both in outline and in internal structure, and Flemming has figured a very similar stage in *Anodonta* (*Entwicklungsgeschichte der Najaden*, Taf. II Figure 32), but each of these authors regards the surface where the polar globule is placed as posterior. The published accounts of the transformation of the glochidium into the adult *Unio* or *Anodonta* (*Ueber die post-embryonale Entwicklung unserer Süßwassermuscheln Anodonta*, von Dr. M. Braun in Würzburg, and: *Zur Entwicklungsgeschichte der Teich- und Flussmuschel* von Carl Scherz), are not sufficiently explicit to decide what the relation between the body of the larva and that of the adult really is, and until some one publishes a satisfactory illustrated account of the transformation, the fact that the vein of the oyster certainly makes its appearance at the point which is occupied by the polar globule must lead us to believe that Flemming and Rafinesque are in error, and that the region between the letters *a* and *i* of Rafinesque's Figure 26, is that which is occupied by the vein in the the marine *Lamell* branch, and therefore the anterior.

The following stage may be reached under especially favorable circumstances within two hours after impregnation, but it is not usual for the embryo to attain to this degree of development in less than twenty-four hours, and it may require more than two days to reach it. The form of the stage now under scrutiny, but after from one to twelve hours the embryo will be found to have assumed the form shown in Figure 33, which is the same view as Figure 32. The outline of the body has now entirely changed, and the anterior end is well rounded and carries the vein, while the dorsal and ventral surfaces converge to the posterior end. The dorsal transverse line in the ventral surface has disappeared, and the transverse line in the dorsal surface has entirely closed, and the notochord has become a continuous rod, thus leaving the embryo as a spherical body of cells inside the body cavity. I was not able to discover any ventral cavity inside the mass, but the cells are so arranged that it would be very difficult to

see a small cavity if one were present, and I do not think there is any reason to believe that the primitive digestive cavity becomes obliterated, although I am certain that this is the fate of its external opening. Before the crescent-shaped transverse groove has entirely disappeared, a small, irregular, transparent body, Figure 36 *s*, makes its appearance at each end of it, and the subsequent history shows that these two bodies are the two valves of the shell, which are entirely separate from each other from the first.

#### THE RATE OF SEGMENTATION.

Before I go on with the description of the later stages of development, I wish to discuss two or three points in connection with the stages which I have already described; one of these is the rate of segmentation.

As I have already stated, the time record which I have given in connection with the figures is exceptionally slow, and I will now give the intervals between certain stages in the development of other lots of eggs for comparison.

**Lot A.**—Warm, bright day. Eggs fertilized at 10 A. M.; segmentation commenced between 12 and 1.30 P. M., averaging about 1 P. M., or three hours after fertilization. The stage shown in Figure 26 was reached by most of the eggs between 2 P. M. and 3 P. M., or about five hours after impregnation.

The stage shown in Figure 32 was reached about 4 P. M., and seven hours after fertilization nearly all the embryos were swimming at the surface.

**Lot B.**—Cool day. Eggs fertilized at 10.30 A. M. About half of the eggs developed, and segmentation commenced between 12.30 and 2 P. M., or about three hours after fertilization. The stage shown in Figure 26 was reached in about twelve hours, and the stage shown in Figure 32 was reached by a very few eggs during the second day, but at the end of the second day all were dead.

Lot C.—Very cold; hail and rain. The eggs from several ripe females were fertilized, but no changes followed, and all the eggs soon decayed.

Another lot of embryos, which were about three days old, and in the stage shown in Plate III, Figure 38, also died.

Lot D.—Day quite cold. The eggs from three females were very carefully fertilized with a mixture of the semen from three males at 10 A. M. About one in one hundred commenced segmentation between 1 and 6 P. M., and developed very slowly. The next day all were dead. As the eggs were perfectly ripe, and became covered with active spermatozoa, their failure to develop must have been due to the low temperature.

Lot E.—Quite cool in the morning; warm and sultry in the afternoon. Eggs fertilized at noon, and segmentation commenced in about two hours. At 7.30 P. M. about half of them had finished segmentation, and at 11.15 P. M. most of them were in the stage shown in Figure 32. On the fourth day most of them were doing well, and had reached the stage shown in Figure 42, when a fall in the temperature killed all of them.

Lot F.—Rather warm. Eggs fertilized at 6 P. M., and the next morning at 5, or eleven hours after fertilization, some were in the stage 32, and some in the stages 36 and 37.

Lot G.—Day quite warm. Eggs fertilized at 8 P. M. At 10.15 P. M., or a little more than two hours after fertilization, nearly all of them were in the early stages of segmentation, and at 5 A. M., or nine hours after fertilization, they were in the stage shown in Figure 37, and in forty-eight hours they were in the stage 42.

Lot H.—Very hot day. Segmentation was completed two hours after fertilization, and in two hours and a half the embryos were in the stage 32, and in forty-eight hours in the stage shown in 43.

I was so far from the water during my investigations that I was not able to make any observations upon the temperature of the oyster beds during the spawning season, but the cases

which I have selected above show the dependence of the young upon continuous warm weather.

The past spring and summer were unusually cool, and it was not until the middle of July that the weather was warm for more than three or four days in succession, and my failure to find any floating embryos in the open ocean may be due to the fact that they were killed by the cold as fast as the eggs were laid. After the middle of July I found a few embryo at the surface of the water of the Sound.

#### EXCEPTIONS TO THE NORMAL METHOD OF SEGMENTATION.

The method of segmentation, as I have described it, is the normal method, and is followed exactly by a very large proportion of the eggs—by more than 90 per cent. of them, I should think; but a few eggs in every lot present considerable variation, especially in the earlier stages.

Plate X, Figures 54 to 62, shows one of the most common variations. If a number of eggs be carefully watched during the early stages a few will be found to reach the stage shown in Figure 13, Plate I, more directly than the ordinary eggs, without going through the process of forming the second micro-mere *b*, of Plate I, and then obliterating it. Figure 54 is an egg two hours and seven minutes after fertilization, and in the stage shown in Figure 4. Two minutes later it had assumed the form shown in Figure 55, which is very similar to Figure 5, except that the three lobes of the trefoil are less sharply separated. Two minutes later it had assumed the form shown in Figure 56, and one minute later the form shown in Figure 57. As shown by these figures, the second micro-mere does not become distinct, as in Figure 6, but the faint indication of it shown in Figure 55 quickly disappears, and the subsequent changes result in the separation of the egg into two masses, instead of three. Figure 58 is forty-five seconds later than Figure 57, and Figure 59 four minutes and fifteen seconds later. A single furrow now extends nearly across the egg, from the polar globule, and divides it into two nearly separated portions—a small one and a large one. In

one minute and a half the two were entirely separated (Figure 60), and in two minutes and fifteen seconds more (Figure 61) each part was prominent and rounded, and in five minutes more (Figure 62) they had again approached each other, and assumed the form of Figure 13. This is the variation which is most frequently met with, and it is plainly a simplification of the normal method, by which the result of the first period of activity is reached more directly. In another variation, which is met with much less frequently, the second period of rest is entirely left out, and the stage shown in Figure 18 is reached directly, as shown in Figures 63 to 66. In this case the egg passes through the stages 1, 2, 3 and 4, of Plate I, but when it assumes the trefoil form of Figure 5, a second plane of cleavage, passing through the axis of the polar globule, divides each micromere into two, as shown in Figure 63, in side view, and in 64, viewed from the formation pole.

Three of these spherules remain distinct, but in a few minutes one of them, which appears to correspond to the second micromere of the normal method of segmentation, becomes fused with the macromere, as shown in Figure 65, which corresponds to Figure 16 of the normally segmenting egg. A few minutes later it assumes the form shown in Figure 66, which is clearly the same as Figure 18. Besides these two variations, which occur quite frequently, and are sufficiently regular to demand especial notice, there are occasional irregularities, such as are always found, when a number of eggs are carefully compared, but these do not call for minute description.

#### THE SIGNIFICANCE OF THE SEGMENTATION OF THE CYSTER EGGS.

Our information regarding the early stages in the development of *Lamellibranchis* is very scanty indeed, but so far as it goes it indicates that the process of segmentation, as I have described it, is, with slight modifications, common to the whole class.

Loven's memoir: *Being til Klæsedommen om Uvecklingen af Mollusca Acephala Lamellibranchiata*. Af S. Loven.

kongl. Vetenskaps-Akademiens Handlingar. Stockh. 1848), contains nearly all the present knowledge of the process of segmentation in the marine Lamellibranchs. I have not the paper before me as I write, and my notes upon it, which were made several years ago, do not contain any figures, so I am not able to make a minute comparison, but the figures which he gives of the segmenting eggs of *Crenella* and *Cardium* are essentially like the same stages in the development of the oyster egg, and show that in these two genera the egg divides into a single macromere situated at the nutritive pole, and a number of smaller micromeres situated at the formative pole, and that the macromere is gradually surrounded by a layer of ectoderm cells, which are formed in part by the division of the micromere and in part by the separation of new micromeres from the macromere.

According to the short abstract which Brobetsky (Studien über die Embryonale Entwicklung der Gasteropoden, von Dr. N. Brobetsky aus Kiew. Arch. f. Mik. Anat. 1870, pp. 95—Taf. viii—xiii) gives of Lovén's observations upon the segmentation of the egg of *Crenella*, p. 104, the resemblance to the oyster does not stop here, but extends to more minute details. The egg divides, as it does in the oyster, into a first and a second micromere, and a macromere which is more transparent than the micromeres. After these three spherules have become distinct, one of the micromeres fuses with the macromere, and all traces of it disappear for a time. The other then flattens down upon the compound mass, and the egg assumes the condition shown in Figure 13. Soon both the first and the second micromeres again become prominent, and then divide, so that there are now four micromeres at one pole of the egg and one larger macromere at the other. One of the four now fuses with the macromere again precisely as it does in the oyster, and a stage like my Figure 19 is reached. As in the oyster, Lovén notices the alternation of periods of rest and activity for some time longer, and the agreement with the oyster appears to be most complete.

Our knowledge of the early stages in the development of the

fresh water Lamellibranchs also shows a great similarity between them and the oyster.

Flemming has given a very minute account of the segmentation of the egg of *Anodonta* (*Studien in der Entwicklungsgeschichte der Najaden*, von Walther Flemming in Prag. mit 4 Tafeln. Aus den lxxi. Bande der Sitzb. der k. Akad. der Wissensch. III Abth. Febr. Heft. 1875. Sitzung am 4. Februar, 1875), and his account of the process (pp. 38-58, and his figures, Taf. II. Figures 1-20), show that the segmentation is much like that of the oyster, except that the segmentation cavity makes its appearance very early.

The segmentation of the egg of the *Anodonta* differs from that of the oyster egg in a number of minute details, but not so much so as to obscure the fundamental similarity. Flemming's Figure 6, of Plate II, obviously represents the same stage as my Figure 13; his Figure 11 is the same as my Figure 19; his Figure 13 the same as my Figure 22; his Figure 17 the same as my Figure 26; his Figure 18 the same as my Figure 28, and his Figure 23 the same as my Figure 32. The polar globules are shown in most of his figures, and prove that, as in the oyster, the growing edge of the layer of ectoderm is at the point which is farthest from these bodies.

The early stages in the development of *Unio* have been figured and described at length by Rabl. (*Ueber die Entwicklungsgeschichte die Malermuschel. Eine Anwendung der Keimblätter-Theorie auf die Lamellibranchiaten*, von Carl Rabl, *Jenaische Zeitschr.*, X, 1876, 310-395; Taf. X-XII), and his account shows a still closer agreement with the oyster than is presented by *Anodonta*.

The segmentation cavity of *Unio* makes its appearance very early, but in other respects there are few differences between his account and my own, except that I have not found in the oyster the two large cells which he says become pushed into the segmentation cavity of *Unio*, and give rise to the mesoderm. In a paper which is now in press, on the formation of the digestive tract in the fresh-water Pulmonates, I have been compelled to call attention to the fact that certain figures in an earlier paper by Rabl, on the development of



Pulmonates, are imaginary and unlike anything in nature, and I therefore take pleasure in stating here that my own work upon the oyster tends to show the perfect accuracy of the observations in the present paper, not only so far as the early stages are concerned, but also as regards the later history of the embryo.

Figure 7, Plate X, of the egg of *Unio* is clearly the same as my Figure 13; Figure 10 is almost identical with 19 of the oyster; Figure 11-14 are very similar to 20-23 of the oyster; Figure 15 differs from 26 of the oyster only in the presence of a segmentation cavity; 17 and 18 are the same as 27 and 28 of the oyster, except that they are not flattened vertically, and his figures 28 and 30 are essentially the same as my 32 and 38.

I have already shown that the stage 13 of the oyster egg, which is usually reached by passage through a number of intermediate stages, and by the formation and obliteration of a third spherule, may be reached by a more direct process, which is exceptional in the case of the oyster. It is interesting to notice that Flemming and Rabl agree that the indirect form of segmentation which is normal in the oyster, is wanting in *Anodonta* and *Unio*, and that this stage is reached directly in a manner which is only occasionally met with in the oyster.

There can be no doubt, that in *Anodonta* at least, the trefoil stage is really wanting, and has not simply been overlooked, for Flemming actually watched and has figured the change of the spherical unsegmented egg into the form shown in his Figure 6.

In addition to the observations above referred to, we have a number of papers which deal with the development of various species of *Cycladidæ*, and contain some observations upon the early stages, but no one has succeeded in getting anything like a complete series of observations, and those which are recorded are not at all in harmony with each other. In his "Contributions to the Developmental History of the Mollusca, No. I, The Early Development of *Pisidium pusillum*," (Phil. Trans. 1875, vol. 165, part I), Lankester gives a

short description, pp. 2 and 3, and a few figures, Plate I, Figures 4, 5, 6, 7, 8, 16 and 17, of the process of segmentation. His observations are very fragmentary and unsatisfactory, but they would seem to indicate that the segmentation is total and regular, and not at all like that of the oyster.

Gamīn reaches a similar conclusion, and says (*Beitrag zur Lehre von den embryonalen Blätter bei den Mollusken. Abst. in Hoffman u. Schwalbe's Jahresberichte. 1. 1872*), that in *Cyclas* the segmentation is total and regular, and results in the formation of a spherical layer of similar cells around a central cavity.

Von Jhering and Rabl, on the other hand, give observations which indicate that the segmentation is, on the whole, like that of the oyster.

Von Jhering says (*Ueber die Ontogenie von Cyclas und die Homologie der Keimblätter bei den Mollusken, Zeit. f. Wiss. Zool. 1876-xxvii*), that although he did not succeed in getting as complete a series of forms as Flemming has figured in *Anodonta*, the stages which he has found show that the process of segmentation takes place about as it does in *Anodonta*, and Rabl says, p. 340, that he has observed two stages in the segmentation of *Cyclas*, and that the mode of segmentation is the same here as in *Unio*. In *Taf. XII, Fig. 58*, he shows one of these stages, which differs from one of the later stages of the segmentation of the oyster egg only in the presence of a large segmentation cavity.

These references, which cover the whole field of our exact knowledge of lamellibranch segmentation, show that probably in the *Cycladidæ*, and certainly in *Unio*, *Anodonta*, *Crenella* and *Cardium*, we have nearly the same mode of segmentation as in the oyster; but that the normal method of oyster segmentation is indirect, and may be simplified occasionally in the oyster, and normally in *Unio* and *Anodonta*, by the omission of many of the stages of the process and the retention of those only which lie in the direct line of development. I have described this process in the oyster with great minuteness, and perhaps with tedious exactness, since I believe that it

is of phylogenetic significance, and indicates the origin of the Class Lamellibranchs.

The distinctive characteristics of this form of segmentation are the very small size of the eggs; the appearance of bilateral symmetry with the first cleavage, and the indication at the same time of the anterior and posterior ends and right and left sides and dorsal and ventral surfaces of the adult; the separation of the egg at the beginning of segmentation into a germinative portion, and a portion which is morphologically comparable, during the process of segmentation, to a food yolk, although it is less granular, both in the oyster and in *Crenella*, than the germinative portion, and at a later stage undergoes segmentation, and forms the wall of the digestive cavity, so that it has none of the physiological characteristics of a food-yolk; and the fact that many of the stages in the process of segmentation have no functional importance and may be suppressed. Outside the Lamellibranchs, whenever we have very small simple eggs among the Mollusca, the embryo is radially symmetrical around a central axis, which passes through the polar globules, and it presents, during the process of segmentation, few points of resemblance to the egg of the oyster at the same period. In support of this statement, I may refer to Lankester's figures of the eggs of Nudi-branchs and Opisthobranchs (*Developmental History of the Mollusca*, Plates 5 and 9), to Fol's figures of the eggs of Pteropods and Heteropods (*Etudes sur développement des Mollusques*. *Arch. d. Zool. exp. et gen.* 1875), to my own observations on the segmentation of the Pulmonate egg (*Studies from the Biological Laboratory of the Johns Hopkins University*, 1879), and to Bütschli's and Lankester's accounts of the segmentation of the egg of *Paludina*, ("Entwicklungsgeschichte von *Paludina vivipara*." *Zeit. f. Wiss. Zool.* 1877, and "On the Coincidence of the Blastopore and Anus in *Paludina vivipara*." *Quart. Mic. Journ.* 1876.)

Rabl has briefly discussed the relation of the bilaterally symmetrical, irregular segmentation of the lamellibranchiate egg, to the regular axially symmetrical segmentation of the egg in most Gasteropods (*Entwicklungsgeschichte der Mal-*

ermuschel, pp. 338-345), and concludes that the first is an adaptational modification of the last, which gives the Lamellibranch an advantage in the struggle for existence, and which has therefore been preserved by natural selection. He says, p. 244: "Die inequale Furchung dem sich entwickelnden Embryo einen Vorthail gewährt, und dass dieser Vorthail um so grösser ist, je frühzeitiger sich eine Ungleichheit in den Furchungsproducten bemerkbar macht."

I think, however, that all the evidence which I have given points towards the conclusion that the peculiar segmentation of the Lamellibranchs is due rather to the retention of characteristics which were adapted to some past condition of things than to direct adaptation to the present conditions of life; and we must therefore look for its origin somewhere else than in the regular radially symmetrical segmentation of the small simple eggs of the Pulmonates. I think that a comparison of my account and figures with the figures and description given by Brobetsky and myself of the segmentation of the egg in those Prosobranchs where the eggs are few in number, large, and contain a large food-yolk which is of physiological importance, will fully support the conclusion that we have here the ancestral form of segmentation, which is retained by the small eggs of the Lamellibranchs.

In a paper entitled "Preliminary Observations on the Development of the Marine Prosobranchiate Gasteropods," (Chesapeake Zoological Laboratory, Scientific Results of the Session of 1878, p. 121), I have given outline figures of a few of the stages in the segmentation of the egg of *Urosalpinx*, Plate 8, Figures 1, 2, 3, 4, 9 and 10, and a reference to these figures will show that there is considerable resemblance between this and the oyster egg. A few small transparent micromeres separate off from the surface of the large food yolk, which occupies the nutritive pole of the egg, and gives rise to a blastoderm which spreads over the surface of the food-yolk; growing at one edge, partly by the division of the micromeres and partly by separation of new ones from the yolk.

From the beginning of segmentation the egg is bilaterally symmetrical, and the general resemblance to the oyster egg is

easily seen, although it is not at all complete or minute, but according to Brobetsky (*Studien über die embryonale Entwicklung der Gasteropoden*, von Dr. N. Brobetsky in Kiew. Arch. f. Mic. Anat. xiii. 1877, pp. 95-170. Taf. viii-xiii), the early stages in the development of *Nassa* are almost exactly the same as those of the oyster. The egg of *Nassa* has a large functional food-yolk, and the blastoderm which surrounds it is not simply an ectoderm, since it gives rise to all the germ layers; but before the differentiation of the spherules at the formative pole has made its appearance, segmentation takes place exactly as it does in the oyster, and the first ten figures of Brobetsky's first plate might have been used, without the least change, to represent the stages of the oyster egg which I have given in my first nineteen figures. I hope to publish soon a short paper, illustrated by a comparative table of outline drawings of the segmenting eggs of various Molluscs, in order to illustrate my conception of their significance, but at present I must refer to the various original papers. A reference to Brobetsky's account and figures will show that his Figure 1, Taf. VIII, is almost exactly like my Figure 4; his Figure 2 like my Figure 5; his Figure 3 like my Figure 7; that his Figure 4 shows the change illustrated more at length in my Figures 8, 9, 10 and 11; that his Figure 5, A and B, shows the same stage as my Figure 13; that his Figure 6 is the stage 15 of the oyster; his Figure 7 the stage 16; and that his Figures 8, A and B, are the same as my Figures 18 and 19. The sections of these stages which Brobetsky gives in Plate IX, indicate that the early appearance of bilateral symmetry in *Nassa* and *Urosalpinx* is a condition of things which has been brought about by the presence of a large food-yolk, which does not undergo segmentation, and this conclusion is confirmed by a comparison with Brobetsky's account of the development of *Natica* and *Fusus*, where a true food-yolk is lacking, and the embryo is radially symmetrical during the early stages.

The facts which I have given in regard to the oyster show that the peculiar early stages of segmentation are of no func-

tional importance, since they may be omitted occasionally in the oyster, and normally in *Unio* and *Anodonta*. In *Nassa* we find them again, but they are here associated with the presence of a food-yolk, and I think we are, therefore, justified in concluding that the one-sided, bilaterally symmetrical segmentation which there is occasion to regard as characteristic of the Lamellibranchs, indicates that the Lamellibranchs are the descendants of an ancestral form, in which the eggs were few, large and provided with a food-yolk; that this has been lost, as the eggs became small and numerous, but that the peculiar form of segmentation which was then necessary has been retained perfectly by the oyster, and incompletely by other Lamellibranchs.

In a paper which was printed several years ago, (*The Affinity of the Mollusca and Molluscoida*, Proc. Boston Soc. Nat. Hist. XVIII, Feb. 2, 1876, pp. 225-235), I called attention to a number of reasons for holding the opinion that the Lamellibranchs must be regarded as a side branch from the main stem, of which the Gasteropods are a much more direct continuation, and that all attempts to trace the phylogeny of the higher Mollusca through the Lamellibranchs to lower invertebrates are erroneous and useless; that the highly specialized "veliger" of the marine Prosobranchs is to be regarded as the proto-mollusc, and that the Gasteropods are descended from this with less modification than the Lamellibranchs. The growth of our knowledge of the invertebrates has furnished us with much more material for comparative study than was available at the time this paper was written, and seems to indicate very clearly that the ciliated embryos of the Echinoderms, Gephyreans, Annelids, Polyzoa, Brachiopods, Rotifera, Molluscs and other invertebrates are, all of them, modifications of a common ancestral type, and that the origin of these great groups is indicated by their embryology.

At the same time the careful comparison of adult animals has directed attention to the fact that, in many cases, those groups in which the structure of a type is reduced to its simplest expression, are not ancestral, but degraded, forms. In

such forms as the *Lernæans* and *Entoconcha* the degradation is due to actual parasitism, but degradation may be effected by any circumstances which diminish the complexity of the environment and thus render a simplification of structure advantageous; and the view that the characteristics which are most distinctive of the *Lamellibranchs* have been produced as adaptations to their sedentary life, and that their remote ancestors were similar to those of the higher molluscs, is supported by ample analogy.

It is clear that as an animal becomes adapted to a sedentary life its diffusion must be provided for by an increase in the number of eggs or embryos, and we can easily see that, if the ancestors of the *Lamellibranchs* were animals which laid only a few large eggs, the gradual acquisition of a sedentary habit would demand a decrease in the size of the eggs, in order to permit an increase in their number.

The evidence which the oyster egg furnishes to show that it was at one time provided with a food-yolk, which has been lost, therefore confirms the view that the *Lamellibranchs* are a degraded or simplified group.

I take the opportunity, while correcting the proofs of this paper, to call attention to a highly interesting discussion of a kindred subject, which is contained in an elaborate paper, by Rabl, on the development of *Planorbis*, in the last number of the *Morphologisches Jahrbuch*, which reached me after this paper was written.

Rabl gives a comparative table of figures, in part original and in part selected, of the early stages in the segmentation of the eggs of a number of *Gasteropods*, *Heteropods* and *Pteropods*, and shows that there is a complete series of forms between the radially symmetrical segmentation of the *Planorbis* egg, and the egg of *Nassa*.

His series of figures fully proves his conclusion that the peculiar segmentation of *Nassa* has been brought about by the gradual localization of a specialized food-yolk, and I think all embryologists will agree with him in holding that his facts show the fundamental similarity in plan of segmentation among the *Gasteropods* and *Pteropods*.

Von Jehring's attempt to show that the early stages in the development of Gasteropods differ fundamentally according as they belong to one or the other of the two *phyla* between which he proposes to distribute them, is thus shown to be absolutely without a basis of fact.

In a foot-note to his paper Rabl says that this similarity of ground-plan does not extend to the Lamellibranchs, but the embryology of the oyster shows that it does.

In the Lamellibranch with which he is most familiar, *Unio*, the process of segmentation is greatly abridged, and its true significance is only seen when it is compared with that of the oyster. This comparison shows that the eggs of Lamellibranchs have passed through a change which is exactly the reverse of the one which Rabl has traced, and the peculiar interest of his paper lies in the fact that while he was tracing the process by which the food-yolk was acquired, I was engaged in tracing the process by which it has been lost, and that the form with which his series ends is the one with which my series begins.

It hardly seems necessary for me to say that I do not wish to be understood to hold that the Lamellibranchs are the descendants of *Nassa*, but simply that their ancestors laid eggs like the eggs of *Nassa*.

#### THE FORMATION OF THE DIGESTIVE TRACT.

At the stage shown in Figures 32 and 33, the primitive digestive tract opens by a wide blastopore, which is situated upon what is to become the dorsal surface; and the outline of the body in front of it is rounded and carries the velum, while behind it the outline is angular and pointed. At the stage shown in Figure 36, the outline of the body is nearly the same, and the external changes are so slight that the side which is below in this figure is at once seen to be the same as that which is below in Figure 32. This surface is still marked by a transverse groove, but the blastopore has closed up completely, and one valve of the shell has made its appearance at each end of the groove. Posterior to the groove the papilla,



*a*, runs backwards and downwards, and in front of it the outline of the body is rounded and bears the velum. The time when the valves of the shell make their appearance varies slightly, but I have seen them when the transverse groove was as well marked as it is in Figure 35, and this fact, as well as the similarity in the outline of Figures 32 and 36, does not seem to leave room to doubt that the shell occupies the position of the blastopore. At the stage shown in Figure 36, the endoderm is a pretty compact mass, separated, around its entire circumference from the body wall, and with no traces of a central cavity, although it is perfectly possible that a small cavity may be present.

Figure 37 is an embryo a few hours older, viewed from the right side, with its dorsal surface uppermost. The shell *s* is much larger than it was at the preceding stage, and is usually quite irregular in outline, although a few embryos were found at this as well as at the stage 36, in which each valve was perfectly regular in outline, pear-shaped, and placed with the narrow end nearest the middle line. A little posterior to the shell is the anal papilla *a*, which now carries a few short, stiff cilia or setae. The relative positions of the shell, anal papilla and velum in the preceding stages seem to show with satisfactory clearness that the side which is uppermost in this figure is that which is below in most of the preceding figures, and which I have called dorsal.

The digestive tract is now much larger than at the preceding stage, and its centre is occupied by a distinct cavity, the wall of which is ciliated. At a point nearly opposite the shell this cavity opens externally by a new opening, *m*, and small particles of food now find their way into the central cavity, where they are kept in rotation by the cilia. At this stage the margins of the opening *m* can be protruded so as to form a projecting sucking disk, by which the embryos adhere to each other and to foreign bodies.

The series of stages which I have figured seems to show that this new opening is almost directly opposite the position which the blastopore occupied at stage 32.

In from two to four days after fertilization, the embryo assumes the form shown in Figure 35, which is a view of the right side. The shell is now large and regular in outline and covers nearly half of the surface of the body. The digestive tract now has two external openings, *ca* and *ma*, which are close together on the ventral surface of the body. In a side view, Figures 35 and 36, it was almost impossible to say whether either or both of these communicate with the digestive cavity, but embryos were frequently found with the two valves of the shell stretched out into the same plane, and with the body pulled up and flattened against the shell, and in a dorsal or ventral view of such an embryo it was easy to see that both openings do communicate with the ventral cavity. Figure 41 is a dorsal view of an embryo at the same stage as Figure 35, but with the valves extended.

The stomach is seen through the shell, and when the animal is in the position it is represented with the head end of the gizzard to the left and the stomach posterior to the head end, the stomach and the gizzard will be seen at the top of the shell, while the intestine *in* is in the centre of the head at the same end.

It will be seen that these openings are much farther apart when the body is extended than the opening of the valves when they are in the extended position, that is, as they are in the side view, Figure 35. The anterior opening, stomach *ca*, is situated lower than the stage, a single tube, but a large, short, spherical pouch, which is easily distinguished from the anterior intestine. I was not able to determine whether these two openings were or were not formed by the division of the single opening shown in the stage 17, and therefore cannot say whether the opening in the figure is the mouth or anus or both.

In an embryo at the same stage (Figures 43 and 44) the gizzard is larger and more rounded considerably in size, and the stomach is more or less in a similar position to the anterior part of the gizzard, as shown in Figure 43. At the stage shown in Figure 44 the gizzard valve is the same as the stage 17, and the

form a pair of pouches or diverticula, the halves of the liver, *l*, in the walls of which numerous highly refractive oil-globules make their appearance.

Our knowledge of the digestive tract in the Mollusca is at present in the greatest confusion; and in most molluscs the difficulties of observation are so great at the time when the most important changes take place, that it is almost impossible to obtain any perfectly satisfactory results.

The oyster presents exceptionally favorable conditions for investigating this question, and the observations above described seem to show conclusively—

1st. That there is an invaginate gastrula stage.

2d. That the blastopore closes completely, leaving the digestive tract without an opening.

3d. That the shell appears at the point which the blastopore previously occupied.

4th. That first one and then two openings from the outer surface into the digestive tract make their appearance almost directly opposite the position of the blastopore, and that one of them becomes the mouth and one the anus.

The most thorough and satisfactory account of the origin of the digestive tract in Lamellibranchs is that of Rabl (*Entwicklung der Malermuschel*), and the process, as he describes it, is like what I have observed in the oyster, so far as all the leading points are concerned. At the close of segmentation the single large macromere of *Unio* divides into a number of large cells, which cover the dorsal surface of the embryo, Taf. X, Fig. 23. They then push into the body cavity, so as to form a primitive digestive cavity, which opens by a dorsal blastopore. Taf. XI, Fig. 28.

The shell now appears, and covers up the dorsal surface, Taf. XI, Fig. 34, and the blastopore closes up, thus leaving the digestive tract without any opening. Taf. XII, Fig. 54.

A new opening is now formed by the invagination of the integument, at a point on the ventral surface, Figure 54, which is at some distance from the blastopore, but not directly opposite it, as in the oyster.

Flemming was not able to obtain much information regarding the history of the digestive tract, but as he has conscientiously adhered, in his description, (*Entwicklungsgeschichte der Najaden*), to what he has been able to actually see, his account agrees with Rabl's so far as it goes, and there is every reason to believe that the process is the same in *Unio* and *Anodonta* and the oyster. Flemming shows that in *Anodonta* the single large macromere divides up into a layer of large cells which occupy the dorsal surface of the body, and subsequently become covered by the shell, but he was not able to trace their future history.

The various accounts of the origin of the digestive tract in the Cycladidae are so very contradictory and irreconcilable that it does not seem worth while to try to get at the truth by comparing them, without making any original observations, and Rabl's paper and my own fairly represent the present state of our knowledge of the origin of the digestive tract in Lamellibranchs. The difficulties of observation are so great that the observations of an investigator who did not direct especial attention to the subject are not likely to afford information of much value, and at the time Lovén's paper was written the problems of invertebrate embryology were so different from those of the present day that no conclusions regarding the history of the digestive tract can be drawn from his figures.

#### THE DEVELOPMENT OF THE OYSTER AND THE GASTRULA THEORY.

Salensky has given (*Bemerkungen über Hæckel's Gastraea-Theorie*. Arch. f. Naturgeschichte, 1874), a very brief account of the origin of the digestive tract in the oyster, illustrated by three figures. He says, p. 150, "das erste Stadium der Entwicklung ein Embryo ist, welcher aus zwei Schichten besteht, und in Inneren keine Höhle trägt," Figure 1, Taf. V, "dass sich dann verschiedene äussere Organe und eine Mundestülpung bilden, und schliesslich im Inneren des Entoderms eine Darmhöhle entsteht," Figures 2 and 3.

After a very exhaustive and able review of the facts in embryology with which we were at that time acquainted, he con-

cludes that the embryology of the oyster and that of other Metazoa proves that the starting point in their development is not the gastrula stage, but a "planula" stage, in which the embryo consists of two layers of cells without a central cavity; that this planula stage may be so modified as to give rise to a "blastula" stage, in which a double layer of cells surrounds a central cavity without an external opening; that while the blastula is to be regarded as a modification of the planula, the planula stage may be omitted, and the embryo may at once assume the blastula form; that the formation of the stomach-cavity is a later and secondary occurrence in the history of development, that it takes place at different stages in different animals, and has no place in a conception of the general plan of development; that either the planula or the blastula may complete its development by passing through a "gastrula" stage, which, however, is not the primitive condition of the embryo, but a secondary modification of later formation, which may or may not present itself; that the "gastrula" stage of development is not the common starting point for all Metazoa; and that the hypothetical "Gastraea" cannot be regarded as the ancestral form from which the higher groups have been derived, pp. 159-173.

A comparison of his figures of the oyster embryo with my own shows that his Figure 1 represents a stage between my Figures 36 and 37; his Figure 2 one at the same stage as my Figure 38, and his Figure 3 one at the stage as my Figure 45.

He is correct in the statement that the embryo shown in his first figure consists of a central mass of endoderm which is entirely surrounded by the ectoderm, and in which no cavity can be seen; and that the mouth and anus are formed and the stomach-cavity becomes visible at a late stage, after the shell and velum have appeared.

My own observations, and those of Rabl on *Unio*, show, however, that the so-called "planula" of the oyster is preceded by a true invaginate gastrula stage, and that, so far as the development hypothesis above quoted rests upon the embryology of the oyster, it is directly opposed to the facts.

Salensky's hypothesis seems to be almost identical with Ray-Lankester's so-called "planula theory," published the year before (*On the Primitive Cell-layers of the Embryo as a Basis of Genealogical Classification of Animals. Ann. and Mag. Nat. Hist.*, May, 1873: and, *Notes on Embryology and Classification, for the use of Students. London: 1877*), the essential difference between which and Haeckel's gastrula theory is the conception of the gastrula as a secondary modification of the planula. The fact that in *Unio* and in the oyster a planula is formed by the modification of a gastrula, would seem, so far as it goes, to be as adverse to Lankester's hypothesis as it is to Salensky's. Von Jhering's view, that the primitive embryonic form among the Mollusca is not a gastrula, but a "leposphaera," does not seem to involve any new conception, for according to his definition (*Ueber die Ontogenie von Cyclos, und die Homologie der Keimblätter bei den Mollusken, von Dr. Hermann von Jhering. Zeitschr. f. Wiss. Zool.* xxiv. Marz. 1876, pp. 414-438), his leposphaera is the same as Lankester's and Salensky's planula. "Die Leposphaera wird also aus zwei concentrischen Zellschichten gebildet von denen die äussere oder das primäre Ectoderm die innere oder das primäre Endoderm umgiebt, wie die Schale einer Nuss den Kern einschliesst. Der bleibende Mund entsteht im Ectoderm der Leposphaera, der Oesophagus entweder vom Munde aus, wie bei den Gasteropoden, oder vom primäre Endoderm aus, wie bei den Lamellibranchien," p. 429.

The facts in the development of the oyster are thus seen to be opposed to the only consistent and probable hypothesis which has ever been proposed in the place of the gastrula theory; the hypothesis that the planula is the primitive embryonic form, of which the gastrula is a specialized modification; but it must not be concluded that since the embryology of the Mollusca is opposed to the alternative hypothesis it therefore tends to support the hypothesis that the gastrula stage has a phylogenetic significance, and shows the descent of the Mollusca from an ancestral gastraea.

The most satisfactory and trustworthy papers which we possess upon the development of the Mollusca show that

while there is in many cases a true gastrula stage, its orifice bears no constant relation to the definitive mouth and anus.

There does not seem to be any valid reason for disputing Fol's statement (*Etudes sur le développement des Mollusques*, I. Ptéropodes, arch. d. Zool. exp. et gen. IV, 1875), that on the Ptéropods the orifice of invagination persists and becomes the definitive mouth. We have the testimony of both Lankester and Bütschli ("On the Invaginate Planula or Diploblastic phase of *Paludina vivipara*," by E. Ray Lankester. Quart. Jour. Mic. Sc. XV, 1875; and "On the Coincidence of the Blastopore and Anus in *Paludina vivipara*," by E. Ray Lankester. Quart. Journ. Mic. Sc. XVI, 1876, and "Entwicklungsgeschichtliche Beiträge. I. Zur Entwicklungsgeschichte von *Paludina vivipara*, von O. Bütschli, Zeitsche. f. Wiss. Zool, XXIX), to prove that in *Paludina* the orifice of invagination persists and becomes the anus.

In the oyster the orifice of invagination closes up, and forms the shell and in the Squid it is perfectly certain that the point where the blastoderm folds in over the food-yolk has no connection with either the mouth, the anus or the shell-gland.

It is not necessary to extend the above list by references to observers who have given still different accounts. There are fine grounds for disputing the correctness of many of these papers, but I do not think the present state of our knowledge gives us any reason for doubting the conclusions of Fol, Lankester and Bütschli; my own observations on the oyster are confirmed by Rabl, and in a future paper I hope to show that the Squid is such a favorable subject for study that there is no chance for error in the statement which I have given above, and we may conclude that the present condition of embryology fully justifies Lankester's statement that in the Mollusca there is no necessary connection between the blastopore and either the mouth or the anus.

Now there cannot be the least doubt that the molluscan mouth is the same opening in all the classes, and that the anus is also homologous throughout the whole group.

- i It is perfectly possible that the mouth and anus might exchange functions during the evolution of a group of animals, or that one or both might be replaced by new openings, and Semper (Stammverwandschaft der wirbelthiere und wirbellosen), und Dohrn (Ursprung wiebelthiere), have given very convincing evidence that such a change has actually taken place in the vertebrate mouth during the evolution of these animals, but there is not the least reason for believing that anything of the kind has taken place during the evolution of the classes of Molluscs, but the whole of the evidence furnished by Comparative Anatomy and Embryology tends to show that nothing of the kind has taken place, but that the mouth and anus, and the shell-gland as well, can be homologized perfectly in all the classes of true Molluscs, and that they are not only homologous with each other, but must be perfectly homologous also with similar structures in the ancestral form of which the classes of Molluscs are modifications.

If there has been a time when all the classes of Molluscs were represented by a single form, a *proto-mollusc*, with a mouth, an anus and a shell-gland, which were homologous with the similar structures in all its descendants, this ancestral form must have been much later than the "gastræa," and if it was produced by evolution from a gastræa at all, it is plain that the mouths, anuses and shell-glands of all the classes of Molluscs must bear the same relation to the organs and openings of this ancestor—the "gastræa."

The fact that the blastopore of the gastrula stage does not, according to our best information, bear any such constant relation to the body of the adult, therefore opposes the conclusion that this stage has a phylogenative significance, and we are fully warranted in the statement that the present state of our knowledge forbids the acceptance of the gastrula theory as an established generalization of scientific value.

I do not think, however, that we are justified in going farther, and concluding the theory is disproved by the facts of molluscan development.

The early stages of the development of the different classes of vertebrates presents, at first sight, few points in common,



yet Balfour has shown (A Monograph on the Development of the Elasmobranch Fishes, by E. M. Balfour, M. A. London: 1878), that the types of the early development of all vertebrate animals can be easily derived from that of the typical gastrula.

The general occurrence of a gastrula stage in so many widely separated animals is certainly the most pronounced feature in embryology, and it is possible that a more complete acquaintance with the development and phylogeny of the Mollusca may show that the facts held do not, in reality, oppose the view that it is an ancestral form, and the conclusion which the facts seem to justify is not that the gastrula theory is proved or disapproved, but that our acquaintance with the facts must be very much greater than it is at present before we shall be prepared to establish any general hypothesis as to the ancestry of the Metazoa.

#### THE SHELL.

The two valves of the shell of the oyster originate separately, as I have already stated, while in some other Lamellibranchs the separation of the valves is brought about at a later stage of development, by the division of a continuous embryonic shell.

In *Cyclas* the embryonic shell makes its appearance as a simple, nearly circular cup, which occupies the dorsal midline of the body, and soon becomes saddle-shaped, and prolonged to form two flaps, which run down into the sides of the body. This embryonic shell does not contain any calcareous matter, but appears to be wholly made up of a chitinous excretion from the cells of the shell area. After it has extended out onto the sides of the body, calcareous matter begins to be deposited on its inner surface, at two points, one on each side of the body. These centres of calcification grow on all sides, and become the calcareous valves of the shell, and the flaps of the primitive shell become the epidermic coverings of the outer surfaces of the two valves. The two centres of calcification grow towards, but not quite up to the medium.

line of the dorsal surface, and these upper edges are united by the middle portion of the primitive shell, which becomes converted into the hinge ligament.

In *Anodonta*, and apparently in *Unio*, the process is somewhat similar, but each calcareous valve is formed by the union of a number of patches which are deposited on the inner surface of the embryonic shell at several centres of calcification.

In the oyster the primitive shell appears to be wanting, and the two valves of the shell correspond to the two centres of calcification which are present in *Cyrtus*.

The fact that the primitive shell of *Cyrtus*, or of *Anodonta*, closely resembles the embryonic shell of a *Chastropoda*, *Pteropoda*, or a *Cyrtopoda* in appearance and shape, as well as in position and method of formation, would seem to indicate that the material with which the shell of *Cyrtus* is formed is the primitive material, and the process in the oyster, a secondary modification.

#### THE MANTLE

The manner in which the lobes and cavity of the mantle are formed in the oyster appears to differ slightly from the process as it exists in *Anodonta*, the dorsal portion of the body-wall being thicker.

In *Anodonta* the mantle cavity is formed by an invagination of the body-wall between the two ventral edges of the valves, and in *Cyrtus* the mantle does not form the sides of the body, as in *Anodonta*, the body being winged. The recess in the oyster is about half-way between that in *Cyrtus* and that in *Anodonta*. The mantle is formed as a ridge or fold of the integument on each side of the body, but this ridge is situated at the ventral edge of the shell, not, as in *Cyrtus*, in advance of it.

#### THE MUSCLES OF THE

The following is a list of the papers in which I have referred to the subject of the present paper:

History of the Oyster and Oyster Fisheries. By I. C. Thompson. London, 1878.

Die Austern und die Austernwirtschaft. von Karl Möbius. Berlin: 1877.

Die danske Osterbanker. H. Kroyer. Kjöbenhavn: 1839.

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## ERRATA.

Owing to an unfortunate oversight the proofs of the last signatures of this paper were not seen by me until after the paper was printed, and I find it necessary to correct some of the most serious of the misprints which have thus found their way into the text.

Page 74, line 16, for *keimblätter* read *Keimblätter*.

- " 75, " 5, " on read in.
- " " " 14, " *Jur* read *Zur*.
- " " " 15, " *zeitsche* read *Zeitschr*.
- " 76, " 4, " *wirbelthiere und wirbellosen* read *Wirbelthiere und Wirbellosen*.
- " " " 5, " *Ursprung wiebelthiere* read *Ursprung der Wirbelthiere*.
- " " " 30, " *phylogenative* read *phylogenetie*.
- " " " 35, " *the theory* read *that the theory*.
- " " " 38, " *presents* read *present*.
- " 76, " 2, " *E. M. Balfour* read *F. M. Balfour*.
- " 78, " 1, " *these* read *their*.
- " 79, " 11, " *Schwalbés* read *Schwalbe's*.
- " " " 31, " *Phila* read *Phil*.
- " " " 36, " *Eraiche* read *Fraiche*.
- " 80, " 5, " *wirbelthiere* read *Wirbelthiere*.
- " " " 18, " *Utricklingen* read *Utvicklingen*.
- " " " 23, " 1870 read 1876.

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## THE DEVELOPMENT OF THE OYSTER.

BY W. K. BROOKS.

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### EXPLANATION OF THE PLATES.

Unless the contrary is stated the figures are drawn with a magnifying power of 250 diameters; Zeiss. F. 2, but it was necessary to amplify the sketches considerably in order to reproduce, by the process of photo-engraving, the features which this magnifying power rendered visible, and the figures as they are reproduced are of about twice the diameter of camera sketches made with the same magnifying power.

The first thirty-two figures show the process of segmentation. Figure 1 is an egg at the end of the first period of rest; Figures 2, 3, 4, 5, 6 and 7, the changes during the first period of activity; Figures 8, 9, 10, 11, 12 and 13, the changes during the second period of rest; Figures 14, 15 and 16, those which take place during the second period of activity; 17, 18 and 19, those which take place during the third period of rest; 20 and 21, during the third period of activity; 22, during the fourth period of activity; 23, during the fifth period of activity, and the remaining figures show more widely separated stages. In all the figures of segmentation, except 29, 30 and 31, the formative pole is above and the nutritive pole below.

PLATE I.

Figure 1.—Eggs two hours and seven minutes after fertilization. It is now perfectly spherical, with an external membrane, and the germinative vesicle is not visible.

Figure 2.—The same egg two minutes later. It is now elongated; one end is wider than the other, and a transparent area at the broad end marks the point where the polar globules are about to appear. At the opposite end the external membrane is wrinkled by waves which run from the nutritive towards the formative pole in rapid succession for about fifteen seconds.

Figure 3.—The same egg two minutes later.

Figure 4.—The same egg two minutes later. The yolk has become pear-shaped. The polar globule has appeared at the formative pole, in the middle of the broad end of the pear, and the nutritive end of the egg is now less granular than the formative end.

Figure 5.—The same egg two minutes later. Three equidistant furrows have made their appearance, separating it into a single mass at the nutritive pole, and two at the formative pole. At this stage the three masses are about equal in size.

Figure 6.—The same egg two minutes later. The first micromere, *c*, is now perfectly separated, and smaller than the second, *b*, and each is smaller than the macromere, *a*.

Figure 7.—The same egg one minute later. Both micromeres are separated and are spherical, as is also the macromere. This stage ends the first period of activity.

Figure 8.—The same egg forty-five seconds later. The two micromeres have begun to fuse with each other, and the second micromere, *b*, is also partially fused with the macromere, *a*.

Figure 9.—The same egg one minute later. The first micromere, *c*, has also begun to unite with the macromere.



# DEVELOPMENT OF THE OYSTER.

Plate I.

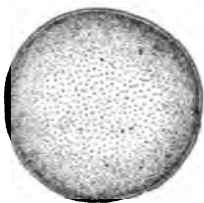


Fig 1.

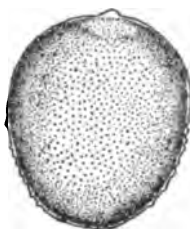


Fig 2.

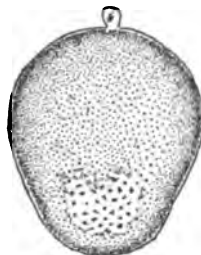


Fig 3.

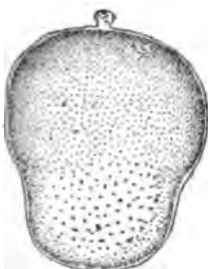


Fig 4.

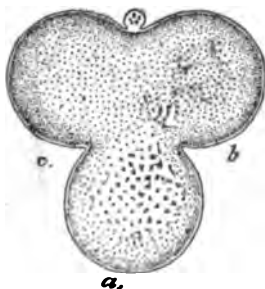


Fig 5.

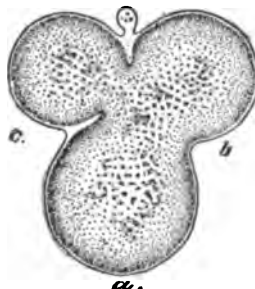


Fig 6.

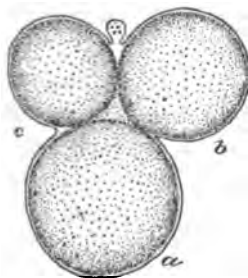


Fig 7.

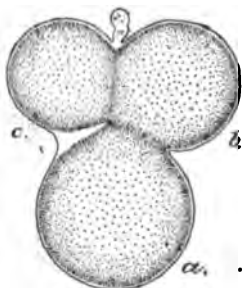


Fig 8.

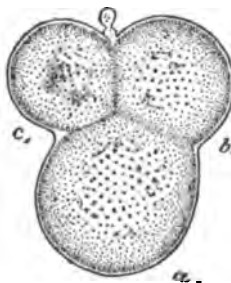


Fig 9.

W. K. BROOKS, Del.





24-71

The first thing I noticed was the time between the two machines will increase the frequency and the distance between them from one end of the spectrum to the other.

The first is — The same old time things were. The second is a kind of new compromise with a more conservative version of what it is the last thing was. And something in the middle.

Page 2 - The end of the second and third sections

Figure 11. — The first two minutes after the start of the test at the end of the second period of rest. The two rats have become startled. The position of an observer standing adjacent to one side of the cage.

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THE UNIVERSITY OF CHICAGO

1. 凡在本行开立存款账户的客户，均可向本行申请开立支票。  
 2. 支票的有效期为自签发之日起六个月内。  
 3. 支票的金额不得超过账户余额。  
 4. 支票的签发人必须为账户持有人或其授权代理人。  
 5. 支票的收款人必须为本行客户。  
 6. 支票的签发必须填写完整，包括日期、金额、收款人等。  
 7. 支票的签发必须加盖预留印鉴。  
 8. 支票的签发必须使用本行规定的支票格式。  
 9. 支票的签发必须使用本行规定的支票用纸。  
 10. 支票的签发必须使用本行规定的支票印章。

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# DEVELOPMENT OF THE OYSTER.

## Plate II.

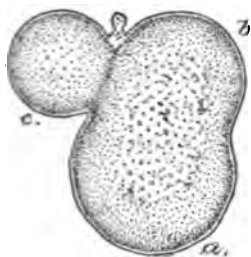


Fig 10.

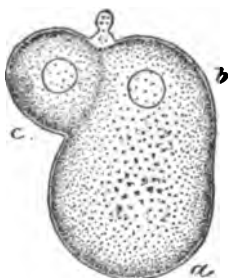


Fig 11.



Fig 12.

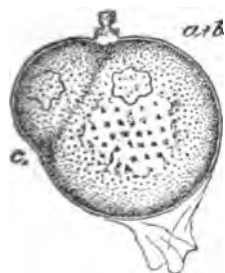


Fig 13.

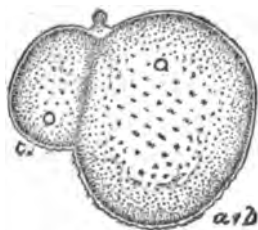


Fig 14.

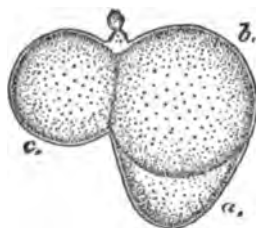


Fig 15.

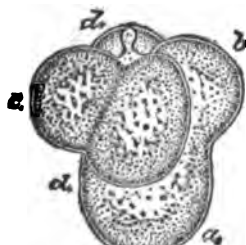


Fig 16.

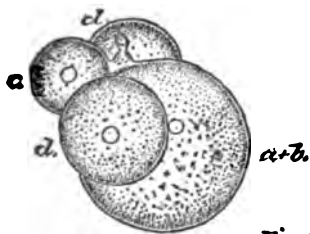


Fig 18.

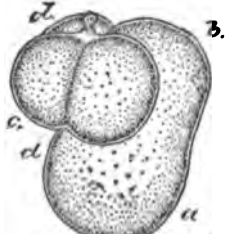


Fig 17.





PLATE III.

Figure 19.—The same egg two minutes and a half later, at the end of the third period of rest, viewed at right angles to Figure 18.

Figure 20.—The same egg thirteen minutes later, and in the same position as Figure 18. The spherule, *c*, of Figure 19, has divided into two, and the second micromere, *b*, has become prominent, so that there are five micromeres at the formative pole.

Figure 21.—The same egg one minute later, and in the same position as Figure 19.

Figure 22.—The same egg in the position of Figure 20, fifteen minutes later than Figure 21, and in the fourth period of activity. There are now seven micromeres at the formative pole, six on one side of the polar globules and one, the second micromere, *b*, on the other.

Figure 23.—The same egg twenty-one minutes later, viewed from the side opposite the second micromere. The cells which have been formed by the division of the micromeres of the stage 19, now form a layer, the ectoderm, which rests, like a cap, on the macromere, *a*.

Figure 24.—The same egg five hours and fifteen minutes later, in the same position as Figure 22, but not quite as much magnified. On one side the polar globule is still separated from the macromere, *a*, by a single spherule—the second micromere, *b*. Opposite this the growing edge, *g*, of the ectoderm is spreading still farther down over the macromere. At the point *g*, and at four other points, are pairs of small cells, which have evidently been formed by the division of the larger spherules.

Figure 25.—Another egg at about the same stage.

Figure 26.—The egg shown in Figure 24, fifty-five minutes later. The macromere, *a*, is almost covered by the ectoderm, and the second micromere, *b*, has divided into a number of spherules. At the growing edge, *g*, an ectoderm spherule is seen separating from the macromere.

Figure 27.—A similar view of an egg twenty-seven hours after impregnation. The macromere is almost covered by the ectoderm, *ec*, and is not visible in a side surface-view. At *g* is an ectoderm spherule, which is separating from the macromere.

Figure 28.—Optical section of the same egg; *ec*, ectoderm; *en*, macromere, divided into two spherules. No segmentation cavity can be seen in a normal egg at this or any of the preceding stages.



# DEVELOPMENT OF THE OYSTER.

Plate III.

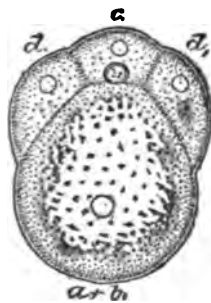


Fig 19

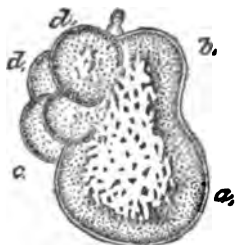


Fig 20.

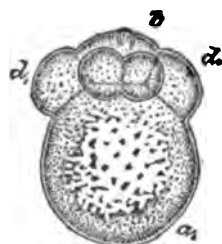


Fig 21.

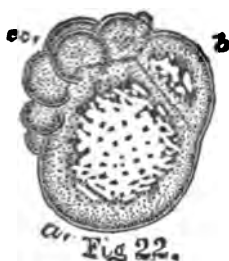


Fig 22.

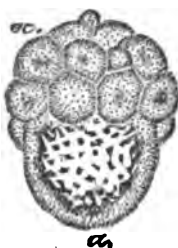


Fig 23

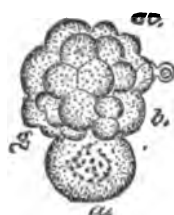


Fig 24.

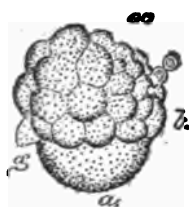


Fig 25.

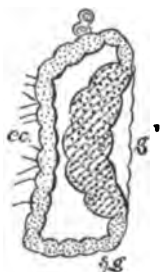


Fig 31.



Fig 26.

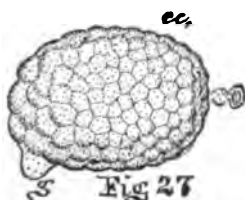


Fig 27

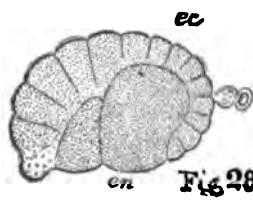


Fig 28.

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PLATE IV.

Figure 38.—View of the primitive pole of an egg a few hours later.

Figure 39.—View of the primitive pole of a still later egg.

Figure 40.—Typical vertical section of a somewhat later egg, figured with the polar globule above and the embryo in the right. The egg is now flattened from above downwards, and is elongated in a slight curve. The macular area given rise to a layer of larger granular cells which are pushed in so as to form a large cup-shaped depression. The more transparent an outer cell now makes a few short cells scattered irregularly, and the two layers are separated from each other by a segmentation cavity. This figure is in Plate III.

Figure 41.—Slight curve, and

Figure 42.—Typical section of the embryo at the first swimming stage. The embryo has folded upon the embryonic axis, and is now a prominent figure in the cavity, with an external opening *a*. The cells of the embryo have now made their appearance at and the area occupied by the polar globule. The vesicle present in the egg from which the figure was drawn, but is now seen in other eggs, and is now in a later stage of another embryo, Figure 43.

Figure 44 and Figure 45.—Two slight curves of the embryo, shown in Figure 42.

Figure 46.—A later embryo, in the same position as Figures 44 and 45. The external opening of the primitive opening has now disappeared, and the two plates of the shell have appeared in the place where it had occupied. The embryo has now appeared in with the embryo, and in external cavity has been seen.

Figure 47.—A somewhat later embryo, figured with the polar globule above. There is a large, central, circular depression, with a small depression by the mouth, *a*, which is almost directly above the primitive opening, the position of which is shown by the shell.

# DEVELOPMENT OF THE OYSTER.

Plate IV.

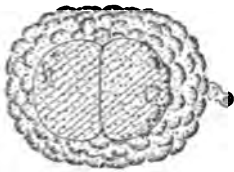


Fig 29.

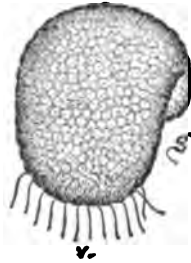


Fig 34.

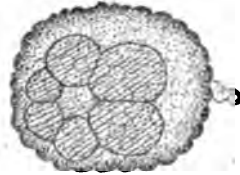


Fig 30

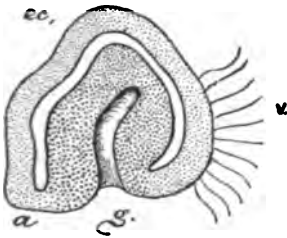


Fig 33.

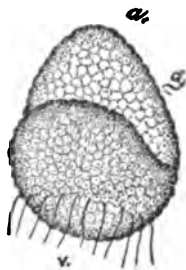


Fig 35.

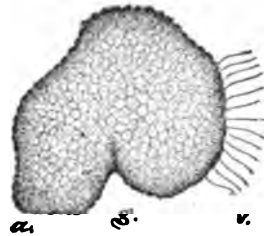


Fig 32.

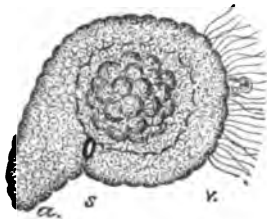


Fig 36.

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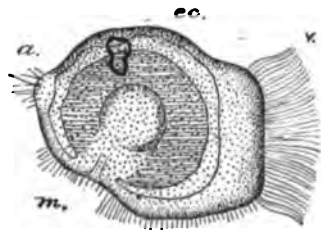


Fig 37.





PLATE V.

Figure 38.—A similar view of a still older embryo. The shell, *s*, has increased in size, and the digestive tract has two openings, the mouth, *m*, and the anus, *an*, which are very near each other on the ventral surface.

Figure 39.—The opposite side of a still older embryo, in which the body-wall begins to fold under the shell, to form the mantle, *m*.

Figure 40.—Dorsal view of an embryo at about the same stage.

Figure 41.—Dorsal view of an embryo at the stage shown in Figure 38, with its valves extended; *rs*, right valve of shell; *ls*, left valve of shell; *an*, anus; *a*, anal papilla; *ma*, mantle; *v*, velum; *b*, body-cavity; *st*, stomach.

Figure 42.—View of left side of a still older embryo; *i*, intestine. Other letters as in Figure 41.



DEVELOPMENT OF THE OYSTER.

Plate V.

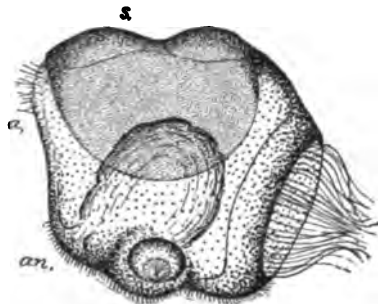


Fig. 38

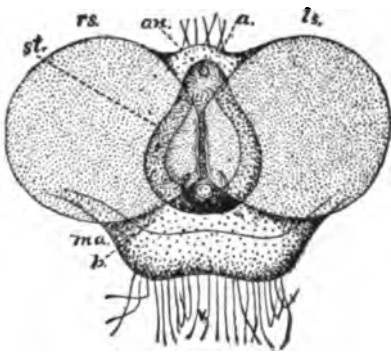


Fig. 41

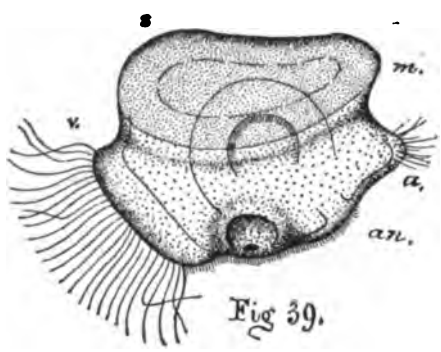


Fig. 39.

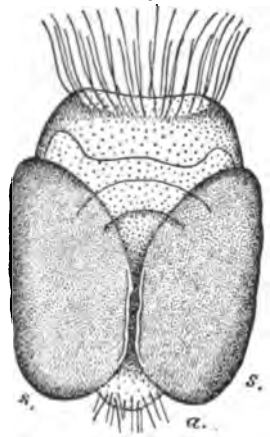


Fig. 40.

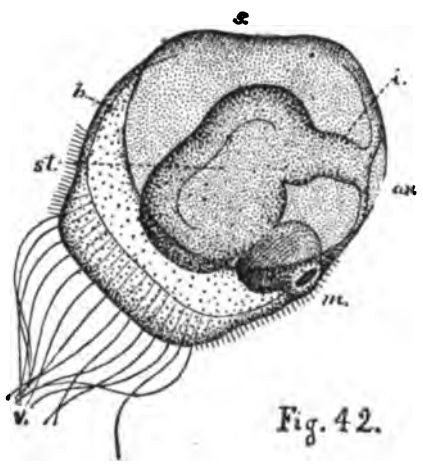


Fig. 42.

W. K. BROOKS, Del.





1. The first step in the process is to identify the problem or issue that needs to be addressed. This involves gathering information and understanding the context of the problem.

2. Once the problem is identified, the next step is to define the objectives and goals of the project. This helps to clarify what needs to be achieved and provides a clear direction for the team.

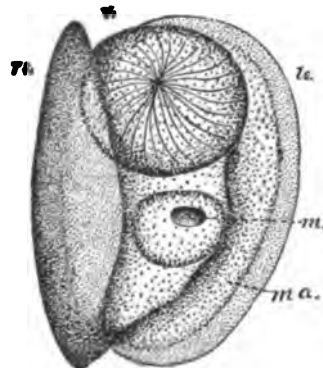
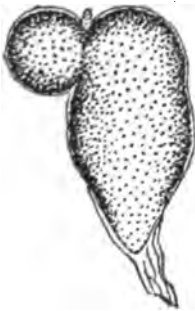
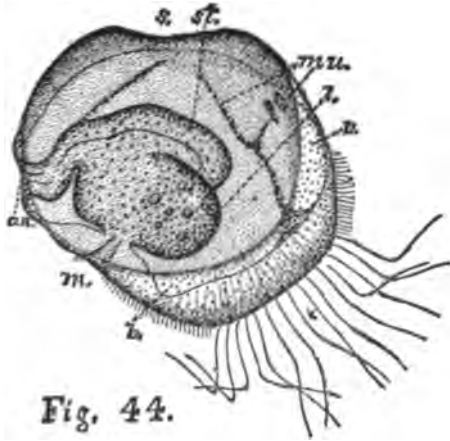
3. The third step is to develop a plan or strategy to address the problem. This involves breaking down the problem into smaller, manageable tasks and determining the resources needed to complete each task.

4. The fourth step is to implement the plan. This involves putting the strategy into action and monitoring progress regularly to ensure that the project is on track.

5. Finally, the fifth step is to evaluate the results of the project. This involves assessing the outcomes against the objectives and goals and identifying any areas for improvement.

## DEVELOPMENT OF THE OYSTER.

## Plate VI.



**W. K. Brooks, Del.**





**PLATE VII.**

**Figure 48.**—The seminal fluid of a ripe male oyster, mixed with water, and seen with a power of 80 diameters. Zeiss. a. 2.

**Figure 49.**—Fluid from the ovary of a ripe female oyster, seen with the same magnifying power.

**Figure 50.**—Seminal fluid of a ripe male oyster, magnified 500 diameters.



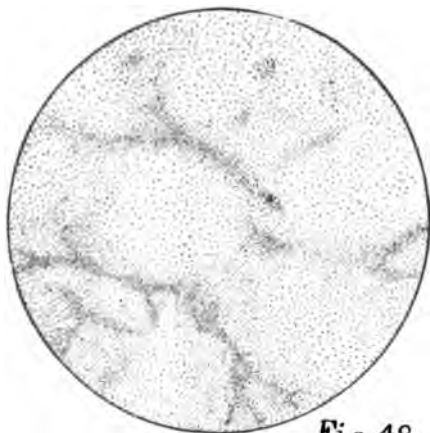


Fig. 48.



Fig. 50.

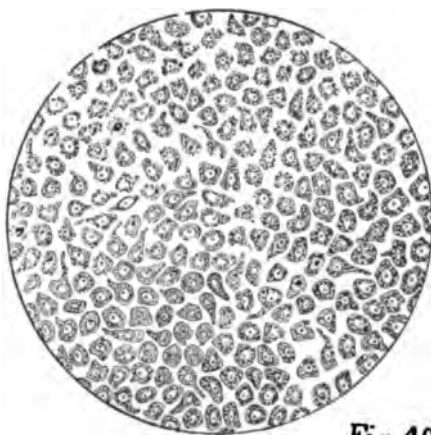


Fig. 49.









PLATE VI.

Figure 43.—Dorsal view of an embryo six days old, swimming by the cilia of its velum.

Figure 44.—View of right side of another embryo at the same stage; *mu*, muscles; *l*, liver. Other letters as in Figure 41.

# DEVELOPMENT OF THE OYSTER.

Plate VI.

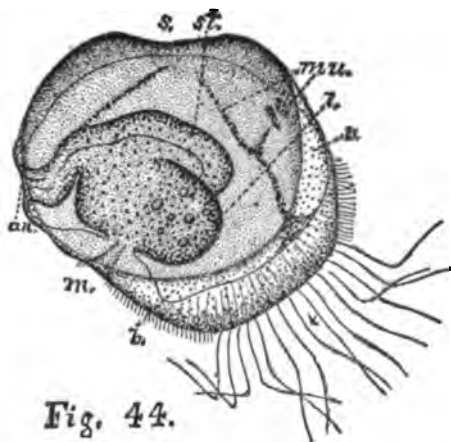


Fig. 44.

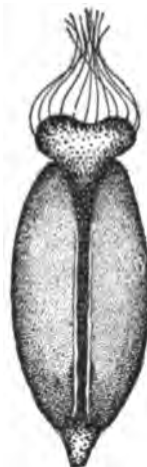


Fig. 43.



Fig. 47.

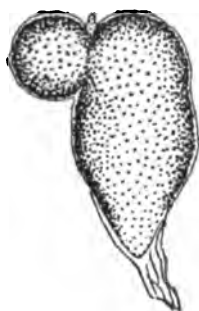


Fig. 46.

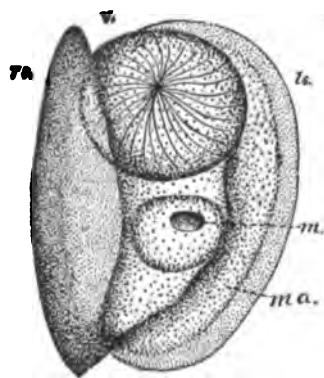


Fig. 45.

W. K. BROOKS, Del.











**PLATE X.**

**Figures 54-66.—Abnormal or direct form of segmentation.**

DEVELOPMENT OF THE OYSTER.

Plate X.



54.



55.



56.



57.



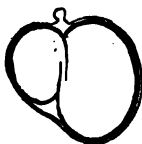
58.



59.



60.



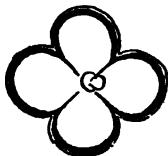
61.



62.



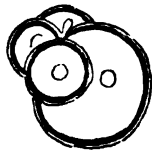
63.



64.



65.



66.









**THE ACQUISITION AND LOSS OF A FOOD-  
YOLK IN MOLLUSCAN EGGS.**

**BY W. K. BROOKS.**



## THE ACQUISITION AND LOSS OF A FOOD-YOLK IN MOLLUSCAN EGGS.

BY W. K. BROOKS.

In a paper on the "Development of the Oyster," which was sent to the printer early in December, 1879, I showed that certain early stages in the segmentation of the oyster egg are of no ontogenetic importance, at present, since they are omitted by a few eggs, which nevertheless complete their development and give rise to perfect embryos. I also showed that the exceptional or direct course of segmentation, which results, in the oyster, from the omission of these stages, is the only course pursued by the eggs of certain other Lamellibranchs, such as *Unio* and *Anodonta*. After calling attention to the fact that a portion of the egg of a Lamellibranch has, during the early stages of development, some of the morphological characteristics of a food yolk, although it soon undergoes segmentation; becomes converted into the endoderm of the embryo; contains less food material than the rest of the egg, and is in no physiological sense a food-yolk; I showed that the egg of the Prosobranchiate Gasteropod *Nassa* presents a form of segmentation which is almost identical, during its early stages, with that of the oyster egg, and that the *Nassa* egg is furnished with a real, functional food-yolk.

As the result of this comparison I concluded that the peculiar segmentation of the oyster egg has an ancestral significance, and indicates that the Lamellibranchs are the descendants of Molluscs which laid eggs furnished with a food-yolk, and that these eggs were large and few in number; that the gradual assumption of a sedentary mode of life, demanding an increase in the number of eggs, gradually brought about a reduction of their size; and that the food-

yolk was gradually lost, although the form of segmentation which was necessary so long as it was present has been retained perfectly by the oyster, and less completely by many other Lamellibranchs.

In the paper referred to I expressed the hope that I should soon be able to publish a second paper, with a comparative table of figures of the series of eggs upon which this conclusion is based; and I am now able to present this table of figures, with such a description as is necessary to show what support they give to my view.

Since the "Development of the Oyster" was written I have received two papers upon closely related subjects. One of these is a German reprint of the well known classic in molluscan embryology, Lovén's "Contributions to our Knowledge of the Development of the Lamellibranchs," with the plates of the original paper, which was published more than thirty years ago, by the Royal Academy of Sweden.

In the preface the author says that he has been induced to publish the reprint by the fact that while great advances have been made in our knowledge of the development of nearly all other groups of Invertebrates, almost nothing has been done upon the embryology of the marine Lamellibranchs. All workers in this field will be pleased to know that they need no longer depend upon unsatisfactory abstracts or upon reference to an almost inaccessible publication in an unfamiliar language, for an acquaintance with one of the most important embryological papers.

In my paper on the Development of the Oyster I stated that while my acquaintance with Lovén's work was unsatisfactory, the information which I possessed indicated that the eggs which he describes undergo substantially the same sort of segmentation as those of other Lamellibranchs. I am now able to state that not only is this the case, but that, of the two forms which he studied, one, *Cardium exiguum*, passes through the abridged or direct form of segmentation, like *Unio* and *Anodonta*, while the other, *Modiolaria marmorata*,

passes through the indirect or ancestral form, and agrees with the oyster in every particular, and resembles the egg of *Nassa* in exactly the same way that the normal oyster egg does.

The second paper: "Ueber die Entwicklung der Teller-schnecke," by Rabl, reached me while I was correcting the proofs of the oyster paper, so that I was able to insert a brief reference to it. Rabl gives a comparative table of figures of the early stages of segmentation of various Gasteropods, Heteropods and Pteropods, and shows that, in these Molluscs, we have a series of eggs which present all the stages in the acquisition of a specialized food-yolk. Starting with the Pulmonate, in which the egg is small, the segmentation total, and the granular food material regularly distributed around the principal axis of the egg; his series ends with the egg of *Nassa*, in which there is a large food-yolk, quite sharply marked off from the protoplasmic germinal portion of the egg. The peculiar interest of Rabl's work, in this connection, lies in the fact that while he was tracing the history of the acquisition of a food-yolk, I was engaged in tracing the stages by which it has been lost, and that the form with which his series ends, an egg like that of *Nassa*, is the one with which my own series begins.

As the relation between Rabl's comparison and my own is shown to the best advantage when the two series are studied together as a whole, I have compiled a table, Plate XI, to illustrate both the acquisition and the loss of the food-yolk.

The figures above the second horizontal line, *b*, illustrate Rabl's view of the manner in which the food-yolk has been acquired, although the forms which I have selected and the stages which I have copied are not precisely the same in all cases as those to which Rabl refers. The series of figures below the first horizontal line, *a*, shows my own view of the manner in which the mode of segmentation presented by the eggs of Lamellibranchs has been brought about by the loss of the food-yolk. The figures in the first horizontal row, *A*, represent stages in the segmentation of *Planorbis parvus*, and are original.

The first figure, 1, is a diagram of an unsegmented egg viewed from the formative pole of the principal axis, and divided into four equal parts by two imaginary planes which pass through this axis. The granular food material is uniformly distributed through these four imaginary segments, and the egg is homogeneous in this view, as well as in a side view, which is given in Figure 2. When segmentation begins the egg divides into two equal and similar spherules, as shown in Figure 3, and then each of these divides into two, so that the egg assumes the form which is shown from the side in Figure 4, and from the formative pole in Figure 5.

After the egg has reached this stage of division into four equal macromeres, the food material in each of them accumulates at the nutritive end, and the formative end becomes more transparent, as shown in Figure 4, and as indicated by the dotted lines in Figure 5. A small micromere now separates from the transparent formative end of each of the macromeres, as shown in a side view in Figure 6.

The second row of Figures, *B*, are stages in the segmentation of the egg of *Planorbis parvus*, after Rabl.

The diagram, Figure 7, is like that of the first series, but a side view of the unsegmented egg, Figure 8, shows that the food material is aggregated at the nutritive end while the formative end is transparent from the first, so that the condition of the food material which is not reached by the egg of *P. parvus* until the egg has been divided into four macromeres is present in the egg of *P. marginatus* before segmentation. With this exception the various stages of segmentation are alike in the two species.

The third row, *C*, gives a diagram of the unsegmented egg of *Cavolinia*, and a side view, after Fol, of the egg at the end of the second segmentation, and in the stage shown in Figures 5 and 10.

The food material is no longer symmetrically arranged around the axis, but has begun to centralize and to leave one segment of the egg comparatively destitute of it. I do not

know whether the unsegmented egg actually exhibits the appearance indicated in the diagram, but at the stage shown in Figure 12 the four macromeres are not alike, but one is small with little granular matter, and the other three large and granular.

The next row, *D*, represents the egg of *Acera*, after Rabl. In the diagrammatic representation the food material is restricted to two segments of the egg, and at the stage shown in Figure 14, which corresponds to Figures 5, 10 and 12, two of the macromeres are small and slightly granular, and two are large and very granular.

The figures in the next row, *E*, are imaginary, or, at least, they have not, so far as I am aware, been figured as actually occurring in the egg of any Mollusc, although I think the actual occurrence of eggs in all the other forms which are represented, warrants the prediction that some of the marine Prosobranchs will in future be found to undergo segmentation in the manner which is here illustrated.

This imaginary egg may be diagrammatically represented as divided into four segments, with nearly all the granular matter localized in one of them, as shown in Figure 15. The first cleavage divides the egg into two unequal spherules, as shown in Figure 16, and the embryo is bilaterally symmetrical from the commencement of segmentation. One of the spherules is small with little granular matter, and the other large and very granular.

The next cleavage, which divides the egg into four spherules, takes place in such a way that the smaller spherule of Figure 16 forms two equal and similar spherules, and the large one forms one small spherule, like the other two, and one which is very large and granular; and the egg assumes the form which is shown in a side view in Figure 17, and in a polar view in Figure 18.

The next row of Figures, *F*, are copied from Brobetsky, and shows the early stages in the very peculiar method of segmentation of the egg of *Nassa*. This egg is very large, and is made up in great part of a true food-yolk, which is

filled with large oil drops, or spherules of deutolycith. After the polar globule has made its appearance at the formative pole of the egg, two slightly granular segmentation spherules make their appearance, simultaneously one on each side of it, and are perched upon the formative end of the food yolk, as shown in Figure 20. Almost immediately after these two spherules have been separated from the food-yolk, one of them again unites with it, and the egg, Figure 21, is now divided into one large spherule with a great quantity of food material and one small, more transparent, spherule, and therefore corresponds to the imaginary egg shown in Figure 16. The spherule which had united with the food-yolk now becomes somewhat separated again, and the egg, Figure 22, is again composed of two slightly granular spherules, perched upon the food-yolk. Each spherule now divides into two, Figure 23, and the egg now consists of four slightly granular spherules, perched upon the food-yolk. One of these four now unites with the food-yolk, and the egg, as shown in a side view, Figure 24, and a polar view, Figure 25, consists of three small slightly granular macromeres, and one very large one, with a rich supply of food, and is like the egg shown in Figures 17 and 18, and differs from those shown in Figures 4, 5, 10, 12 and 14 simply in having one of the macromeres specialized as a food-yolk. Four micromeres, Figure 26, now appear at the formative pole, and the embryo which is gradually formed encloses the greater part of the large macromere in its body-cavity, where it is gradually assimilated as food, and is not directly converted into any part of the embryo.

This egg may be represented diagrammatically, Figure 19, as having nearly all its food matter localized in one of the four segments, like the egg shown in Figure 15, but the localization has gone one step farther, and the germinal protoplasm of this segment is separated from the nutritive portion, so that the egg may be represented as a blastoderm, ideally divided into four parts, and a distinct, local food-yolk. The blastoderm divides first into two segments, as in Figure 20, and



then into four, as in Figure 23, but each period of activity is, in this as in other Molluscs, followed by a period of rest, during which the independence of the separate spherules becomes more or less completely lost, and during this period of rest the characteristic which was most recently acquired, the separation of the nutritive and germinative portions of the nutritive quarter of the egg, becomes completely obscured. Accordingly the stage shown in Figure 20 is followed by a stage, Figure 21, which is like the first stage of segmentation, Figure 16, of an egg in which the last step in the localization of the food-yolk has not been acquired, and the stage, shown in Figure 23, is followed by a resting stage, which is like the second stage of segmentation, Figure 17, of a somewhat less specialized egg. The stages 21 and 24 of the *Nassa* egg are thus shown to have an ancestral significance, and to be due to the fact that a distinct separate food-yolk has been acquired by an egg which was, at one time, like that shown in Figure 15.

The next row of Figures are copies of original drawings, to show the normal method of segmentation in the oyster.

The egg is very small, quite transparent, and, as shown in Figure 27, uniformly granular. After the appearance of the polar globules it divides into three spherules which are about equal in size: two of them are at the formative end of the egg, and one at the nutritive end. The latter corresponds to the food-yolk of *Nassa*, so far as its morphology is concerned, but it is less granular than the other two, and is in no physiological sense a food-yolk. It soon fuses with one of the macromeres, as shown in Figure 29, and the succeeding stages of segmentation, shown in Figures 30, 31, 32 and 33, are almost exactly like the corresponding stages of *Nassa*.

Lovén's figures of the segmentation of the egg of *Modiolaria marmorata* are like those given in the line *G* in every particular, and might have been substituted for them.

The next line, *H*, gives a form of segmentation which is occasionally met with in the oyster. The egg, after the appearance of the polar globule, assumes a trefoil shape, Figure

34, but before it becomes divided into three portions, two of these unite, so that the stage 35 is reached more directly than in the normal egg. Each of the spherules of Figure 35 now divides into two, and the stage 36 and 37 is thus reached directly, by the omission of the stages 30 and 31.

This form of segmentation, which is exceptional in the oyster, is normal in *Cardium exiguum*, but is a little more simplified, as shown in the series *I*, after Lovén. The trefoil stage is barely indicated, for a brief period, by a triangular outline, 39, and the egg divides, at once, into two spherules, and then into four, as shown in Figures 40, 41, 42 and 43.

The next row of figures, *J*, after Flemming, show that in *Anodonta* the trefoil stage is entirely wanting, and the egg divides directly into two and then into four spherules. One of these is much larger than the others, but the segmentation is as direct as it is in *Planorbis*.

This series of figures speaks for itself, and I do not see how the conclusion that we here have the stages in the acquisition and loss of a food yolk can be doubted.

I trust that no one will understand that I hold the oyster to be the descendant of *Nassa*, or *Nassa* the descendant of a Pulmonate.

What I do hold, and believe to be fully proved by this series, is that the egg of *Nassa* has been produced by the gradual modification of such an egg as is laid at present by the Pulmonates, and that the peculiar segmentation of *Nassa* has been caused by the addition of a food-yolk to an originally simple egg; that the egg of the oyster is derived, by slight modification, from an ancestral egg, which was like that which *Nassa* lays at present, and that the segmentation of the eggs of various Lamellibranchs exhibit proof of their descent from large eggs with a true food-yolk.

In a foot note to his recent paper, p. 580, Rabl says that while the Gasteropods exhibit a fundamental similarity of plan of segmentation, there is no agreement between the plan of segmentation of a Gasteropod egg and the egg of a Lamellibranch, and in proof of this statement he refers to his

paper on the development of Unio. The segmentation of Unio is substantially like that of Anodonta, and a comparison of this egg with that of Planorbis will, of course, fail to exhibit any close resemblance, for these two forms are at the ends of a long but tolerably complete series. When Unio is compared with the oyster; the oyster with Nassa; Nassa with Cavolinia, and Cavolinia with Planorbis, the fundamental similarity of plan at once becomes apparent.

W. K. BROOKS.

BALTIMORE, *Feb. 25th, 1880.*

THE ACQUISITION AND LOSS OF A FOOD-YOLK  
IN MOLLUSCAN EGGS.

EXPLANATION OF THE PLATE.

Figures 1 to 6. Diagram and stages of segmentation of *Planorbis parvus*. (Original.)

Figures 7 to 10. Diagram and stages of segmentation of *Planorbis marginatus*. (Rabl.)

Figures 11 to 12. Diagram and stage of segmentation of *Cavolinia*. (Fol.)

Figures 13 to 14. Diagram and stage of segmentation of *Acera*. (Rabl.)

Figures 15 to 18. Diagram and stages of segmentation of an imaginary egg.

Figures 19 to 26. Diagram and stages of segmentation of *Nassa*. (Brobetsky.)

Figures 27 to 33. Normal segmentation of oyster egg. (Original.)

Figures 34 to 37. Direct segmentation of oyster egg. (Original.)

Figures 38 to 43. Segmentation of *Cardium exiguum*. (Lovén.)

Figures 44 to 47. Segmentation of *Anodonta*. (Flemming.)

THE ACQUISITION AND LOSS OF A FOOD-YOLK.

Plate XI.

